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# Efficacy of Prunus Armeniaca on Oral Squamous cell carcinoma Cell Line: An Ex- Vivo study

**Ahmed Fahmi** ( ahmednfahmi@gmail.com )
Beni-Suef University
**Maha Abdelkawy**
Beni-Suef University

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# Abstract

Prunus Armeniaca *(Egyptian apricot)* is the plant of Rosaceae family which is widely known for its fruit. It has high nutritional value as it contains essential amino acids, proteins, carbohydrates, and lipids. Kernel oil extracted from it was found to have antibacterial, antifungal, antioxidant, and anti-inflammatory effects. Its therapeutic effect is still under research. Apricot extracts were found to inhibit in vitro growth of breast cancer and hepatocellular carcinoma.

**Objectives**: Evaluating the effect of Prunus Armeniaca (PA) extract on Oral Squamous cell carcinoma (OSCC)

**Methods**: Egyptian PA seeds were macerated and added to 90% methanol for 7 day for alcohol extract preparations. OSCC cell line of human tongue were cultured and divided onto 2 groups. *Group – 1* was treated with the PA alcohol extract. *Group-2* were untreated cell line of the OSCC human tongue as a negative control group. Caspase-3 and – 8 activity assay, Cell viability and cytotoxicity, and Cell cycle analysis were performed.

**Results**: *Group-1* showed significant increase in caspase-3 & -8 than *group 2*. *Group-1* showed significant minimal cytotoxicity in comparison to Staurosporine. Cell cycle analysis revealed cell growth arrest in S-phase for *group-2* with total apoptosis 7.7 folds than *group-1.*

**Conclusion**: PA extract yields anti-growth properties on human tongue OSCC cell line.


# Introduction

Prunus Armeniaca (Egyptian apricot) is a plant that belongs to Rosaceae family. Its famous for its fruit and is highly produced in temperate areas. It is considered a traditional medicinal plant that is rich in amygdalin (cyanogenic glucoside,), as a major component as well as saccharides, sterol derivatives, metals /minerals, polyphenols, fatty acids, and carotenoids. (Fratianni et al., 2018)

PA, as a member of the apricot family, with its different derivatives and by-products were found to have numerous pharmacological properties including antimicrobial, anti-inflammatory and antioxidants as well as its role in nutrition with its rich nutritional factors (Fratianni et al., 2018, Cassiem & de Kock 2019)

In 2013, Gomaa revealed the significant antibacterial, antioxidant as well as antitumor effect of sweet and bitter apricot kernel. She suggested that apricot could be a new medicinal natural product (Gomaa, 2013). Its essential oils were found to have potent antiproliferative action for cultured human epidermal keratinocytes in vitro and sequentially was thought to have therapeutic effect in psoriasis (Fratianni et al., 2018)

Oral cancer is a common type of cancer where it represents half head and neck cancers. Oral squamous cell carcinoma(OSCC) is the most common type occupying nearly 90% of all oral censers. It shows poor prognosis with high mortality rate especially with late stage discovery (Vitório et al., 2020)

Despite being used frequently as traditional medicine, the anticancer actions of PA hasn't been investigated enough. In 2019, a research was performed by Mahmoudi et al. that studied the anticancer effect on breast cancer.

The therapeutic actions of PA is important but still under investigation as its not supported by enough evidence-based studies. Thus, the aim of this study is to evaluating the effect of PA extract on OSCC(Mahmoudi et al., 2019)

## Methods

# Cell culture

OSCC cell line (Scc-25) (ATCC® CRL-1628™)of human tongue were cultured in 10 ug/ml of insulin (Sigma), DMEM containing 10% FBS, and 1% penicillin-streptomycin at 37°C in a humidified 5% CO2 atmosphere for 24 h. Detachment of adherent cells from 90-mm dishes was performed using trypsin, then for the experimental reasons the cells were seeded in 96-well or 6- well plates

# PA extract preparation

Egyptian PA were purchased from Giza governorate in May 2020, PA seeds were separated, macerated and added to 90% methanol for 7 day for alcohol extract preparations. The solution was later filtered through a gauze, repeatedly for three times, producing clear methanolic extract. Amore effective extract type was chosen according to the cell viability percentage and IC50 value for additional testing

# Grouping:

Cultured OSCC cell line were divided onto 2 groups.

*Group − 1* was treated with the PA alcohol extract.

*Group-2* were untreated cell line of the OSCC human tongue as a negative control group.

# Tests performed:

Caspase-3 and − 8 activity assay, cell viability and cytotoxicity, cell cycle analysis, apoptosis versus necrosis and mitochondrial membrane potential (ΔΨm) assay.

# Caspase-3 and − 8 activity assay

Real-time PCR was used to detect caspase 3 and 8 expression. 3x106 OSCC-25 cells/well were seeded in 6-well culture plates and incubated as indicated. By using RNeasy Mini Kit (QIAGEN), RNA isolation and reverse transcription were done. Reverse transcription and amplification were performed using BIORAD iScriptTM One-Step RT-PCR Kit. Gene- specific primers used were as follows: Casp 3 F 5'-ctcggtctggtacagatgtcga-3', Casp 3 R 5'-catggctcagaagcacacaaac-3', Casp 8 F 5'-ACAATGCCCAGATTTCTCCCTAC-3', Casp 8 R 5'-CAGACAGTATCCCCGAGGTTTG-3', CASP9-F 5'- TCA GTG

ACG TCT GTG TTC AGG AGA – 3', CASP9-R 5'- TTG TTG ATG ATG AGG CAG TAG CCG – 3', β-actin F 5'-GTGACATCCACACCCAGAGG-3' and β-actin R 5' ACAGGATGTCAAAACTGCCC-3'. The assays were completed in monoplicate, and the expression was calculated based on ΔΔCt method. The n-fold change in mRNAs expression was determined according to 2 – ΔΔCT method.

# Cytotoxicity

PA cytotoxicity was compared to the chemotherapeutic agent staurosporine to detect it maximum inhibitory concentration.

# Mitochondrial transmembrane potential (ΔΨm) assay

In a warm culture medium ,1.2x104 OSCC-25 cells/ well were plated to a 96-well plate and incubated overnight then treated as indicated. 10 µl of 2 µM TMRE Labeling Solution was added and placed in an incubator (37°C and 5% CO2) for 20 min. washed 3times with warm 1X PBS. The fluorescent intensities were measured using BD Facscalibure (excitation about 550 nm and emission about 580 nm). (4)

# Statistical analysis.

Data were collected as the mean ± s.e. (n = 3) of Relative fluorescence units (ΔRFU) or relative to ΔRFU of the untreated cells for Δϕ m assay, and of the relative optic density of group I to group II for cell viability and cytotoxicity assay, then tabulated and analyzed.

## Results

# Caspase-3 and – 8 activity assay:

Group-1 showed significant increase in caspase-3 & -8 than group-II (2.972 folds and 2.165 folds respectively)

# Cell cycle analysis

Cell cycle analysis revealed cell growth arrest at S- phase for group-1 with total apoptosis 7.7 folds than group-1.(Fig. 1)

# Apoptosis &Necrosis

PA effect on OSCC led to increase in the apoptosis 7.8 folds than the control group. It also induced necrosis but with only 4 folds in comparison to control group

# Cytotoxicity: using reference drugs Straurosporine

PA cytotoxicity was found to be minimal in comparison to the chemotherapeutic agent Staursporine where its cytotoxicity IC50 was 81 ug/ml while that of Straurosporine was 6.83 (Fig. 2). *Group-1* showed significant minimal cytotoxicity in comparison to Staurosporine

## Discussion

PA seeds have various pharmacological properties, including anti-oxidant, anti-inflammatory, antimicrobial, and anti-cancer activities. It has been used for some dermatological diseases as acne vulgaris, and dandruff as traditional medicine. Its essential oils have been used in vitro acting as a powerful antiproliferative agent for cultured human epidermal keratinocytes, representing a noteworthy aid for psoriasis (Fratianni et al., 2018)

Recent cancer treatment agents are being obtained from plants including fruits, leaves and flowers as well as fungi. Current research is suggesting that plants and herbs do play a vital role in preventing and treating some diseases. These types of cures are known to public as herbal medicines and are being used for their powerful natural compounds. (Levitsky & Dembitsky, 2014)

These different therapeutic properties of PA led us to our research question weather PA have anti cancerous effect on OSCC. Our study included group I; representing the PA group and group II; acting as a negative control group of OSCC human tongue cell line.

Our results showed increased apoptosis in the OSCC cell line treated by PA in comparison to the negative OSCC control group. This was seen through caspase 3 and caspase 8 assays indicating activation of both extrinsic and intrinsic pathways of apoptosis. The cell cycle analysis performed in our study showed cell growth arrest at S phase for OSCC group treated with PA, permanently ceasing the cell growth and inducing apoptosis 7.8 folds more than the untreated OSCC cell line. Furthermore, necrosis increased in PA group 4-fold more than the untreated group. The mitochondrial membrane potential was decreased to 33.79% in PA group in comparison to 66.21% in the untreated group, inducing loss of cell viability which is highly targeted in cancer cells.

We found that PA had low cytotoxicity in comparison to Straurosporine with its least effect dose causing cytotoxicity to br 81ug/ml in comparison to Straurosporine a chemo therapeutic agent, which was found to have IC50 6.83ug/ml. IC50

Apoptosis is a unique form of cell death where a cascade of reactions occur leading to discarding unhealthy or unnecessary cells during development or cellular stress. The expression of some genes that inhibit apoptosis led to promoting cell survival. Hence recent cancer research is directed towards dysregulation of apoptosis being the beginning of carcinogenesis. Number of studies revealed the significant role that apoptosis play in tumorigeneses (Mahmoudi et al., 2019)

Minaiyan et al., performed a study on rats, where they induced ulcerative colitis and treated it with PA comparing it with prednisolone as the standard treatment. Their results showed improve effect in the PA group especially with those treated intraperitoneal than those treated orally. They credited this outcome to the increased bioavailability of the active component on injection (Minaiyan et al., 2014)

In accordance with our results, Mahmoudi et al in 2019, studied the effect of PA on expression of the Bax (Bcl-2-associated X) and c-FLIP (Cellular FLICE-inhibitory protein)genes which are highly expressed in human breast cancer. PA significantly decreased the Bax and c-FLIP genes expression. Consequently they

suspected that PA could be related to anti-apoptotic genes inhibition in Human breast cancer. (Mahmoudi et al., 2019)

An animal study performed on rabbits contradicted our results and stated that the apricot seeds contents, amygdalin, might have a potential risk on animal health as it caused microscopic structure changes in their livers. Nevertheless, this study couldn't verify the toxic effect in relation to the highest dose supplied (Kolesárová et al., 2020)

On the other hand, Cassiem & Kock in 2019 reported that there was an intra S-phase block in the cell cycle of human colon cancer cells in vitro after been treated with South African kernel extract. Amygdalin was found to low the ATP levels causing induction of Pycnosis or necrosis. They concluded that human cancer cells are considered to have different levels of certain enzymes that make them more susceptible to have arrested growth when exposed to amygdalin than normal cells. They suggested that these extracts may play an important role as chemo-preventive agents. (Cassiem & Kock in 2019)

# Conclusion & Recommendations

PA extract induced apoptosis through intrinsic and extrinsic apoptosis, caused cell cycle arrest at s-phase and. **this** implies that PA extract yields anti-growth properties on human tongue OSCC cell line. It's considered a good start for further studies on PA as an anti-cancerous agent.

# Declarations

### Ethics approval

This study has been approved by FD-BSU Research ethics committee given the following number (FDBSUREC/11022021/FA)

### Consent for publication

Both Author consented publication

### Consent to participate

Not applicable

### Acknowledgment

Not applicable

### Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

### Competing interests

There is no competing interest

### Funding

Self-funded

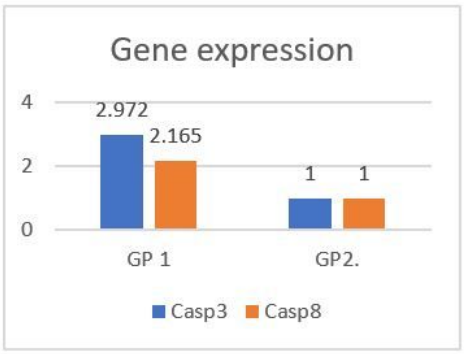
### Authors' contributions

Fahmi A. conceived the study, participated in the design and performed the test. Abdelkawy M. participated in the design and wrote the article

## References

1. Cassiem, W., de Kock, M. The anti-proliferative effect of apricot and peach kernel extracts on human colon cancer cells in vitro. *BMC Complement Altern Med* 19, 32 (2019). https://doi.org/10.1186/s12906-019-2437-4.
2. Florinda Fratianni, Maria Neve Ombra, Antonio d'Acierno, Luigi Cipriano, Filomena Nazzaro, Apricots: biochemistry and functional properties, Current Opinion in Food Science, Volume 19, 2018, Pages 23–29, ISSN 2214–7993. doi.org/10.1016/j.cofs.2017.12.006.
3. Gomaa, E.Z. *In vitro* antioxidant, antimicrobial, and antitumor activities of bitter almond and sweet apricot (*Prunus armeniaca* L.) kernels. *Food Sci Biotechnol* 22, 455–463 (2013). https://doi.org/10.1007/s10068-013-0101-1
4. Kolesárová, A.., Džurňáková, V. ., Michalcová, K. ., Baldovská, S. ., Chrastinová, Ľubica, Ondruška, Ľubomír ., Jurčík, R. ., Tokárová, K. ., Kováčiková, E. ., Kováčik, A. ., & Massányi, P.. (2020). THE EFFECT OF APRICOT SEEDS ON MICROSCOPIC STRUCTURE OF RABBIT LIVER. *Journal of Microbiology, Biotechnology and Food Sciences*, *10*(2), 321–324. https://doi.org/10.15414/jmbfs.2020.10.2.321-324.
5. Levitsky DO, Dembitsky VM. *Anti-breast Cancer agents derived from plants. Nat Products Bioprospect.* 2014;*5:1 Springer.*
6. Mahmoudi, E., Abolfathi, M., Hassanzadeh, N. *et al. Prunus armeniaca* effects on expression of genes related to apoptosis in human breast cancer cells. *transl med commun* 4, 5 (2019). https://doi.org/10.1186/s41231-019-0036-5.
7. Minaiyan M, Ghannadi A, Asadi M, Etemad M, Mahzouni P. Anti-inflammatory effect of Prunus armeniaca L. (Apricot) extracts ameliorates TNBS-induced ulcerative colitis in rats. Res Pharm Sci. 2014 Jul-Aug;9(4):225–31. PMID: 25657793; PMCID: PMC4314870.
8. Vitório JG, Duarte-Andrade FF, Dos Santos Fontes Pereira T, Fonseca FP, Amorim LSD, Martins-Chaves RR, Gomes CC, Canuto GAB, Gomez RS. Metabolic landscape of oral squamous cell carcinoma. Metabolomics. 2020 Sep 30;16(10):105. doi: 10.1007/s11306-020-01727-6. PMID: 33000429.

# Figures




| Sample data | Results DNA content | | | |
|---|---|---|---|---|
| code | %G0-G1 | %S | %G2/M | %Pre-G1 |
| Gp I/SC25 | 47.23 | 51.26 | 1.51 | 18.06 |
| Gp II/SC25 | 51.25 | 43.61 | 5.14 | 2.32 |

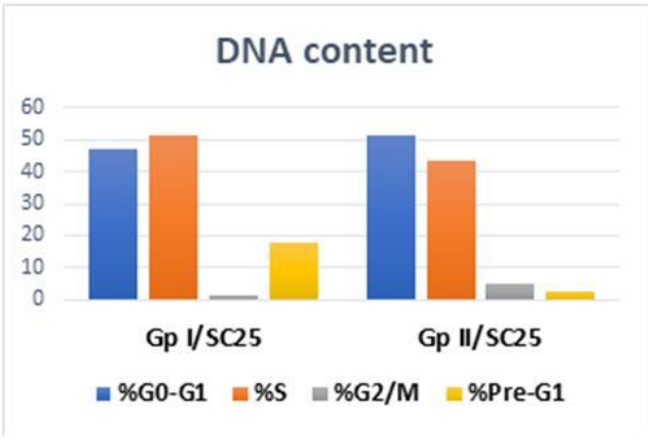
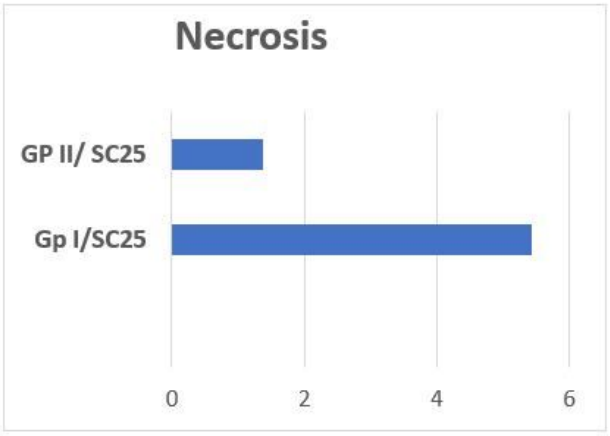
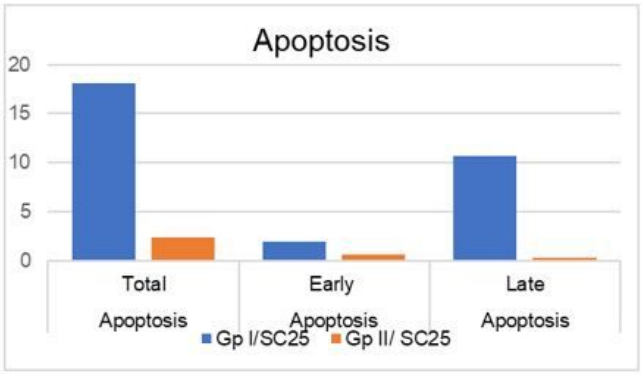


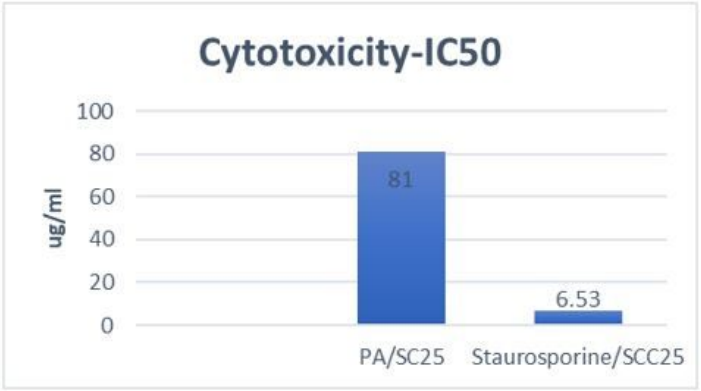

**Figure 1**

showing the results of cell cycle analysis of group 1 & group II with an illustrating bar chart




| | Apoptosis Total | Apoptosis Early | Apoptosis Late |
|---|---|---|---|
| Gp I/SC25 | 18.06 | 1.91 | 10.71 |
| Gp II/ SC25 | 2.32 | 0.61 | 0.33 |




## Figure 2

Results of cytotoxicity of the PA in comparison with staurosporine