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ANTICANCER ACTIVITY OF AMYGDALIN EXTRACTED FROM LOCAL APRICOT KERNELS

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ABSTRACT

The amygdalin was extracted aqueously from the kernels of local apricots at two temperatures (37, 100) C using citric acid. The percentage of amygdalin extracted at 100 C and 37 C was (68.2) and (20.3) ppm, respectively. Amygdalin was used for the purpose of studying its anti-cancer activity using the MCF-7 breast cancer cell line, compared with the liver cell line (WRL-68). Cell viability at a concentration of 25 mg/ml was (82.8) for MCF-7 breast cancer cells compared to the liver cells (WRL-68) was (94.2) at the same concentration. (IC₅₀) for MCF-7 breast cancer cells was 82.74, and 133.9 for liver cells (WRL-68).

Keywords : Amygdalin, apricot kernels, anticancer.

Introduction

The apricot kernel contained within the seeds is characterized by its medical and therapeutic importance as it is rich in primary metabolic compounds (proteins, fats, carbohydrates) and secondary metabolic compounds (cyanogenic glycosides such as amygdalin), as amygdalin is found in the apricot kernel by 3-4% and may rise to 8% (Polarinwa *et al.*, 2014). The compound amygdalin has received more attention in recent years due to its antitumor and anticancer effect, and numerous studies have shown its therapeutic effects on breast, lung, bladder, prostate, and colorectal cancers. But it is controversial because as is degraded by β -glucosidase, a hydrolytic enzyme presents in the human digestive system. In addition to its presence in the cyanogenic glycosides tissues to produce hydrocyanic acid, which later gives cyanide, the compound responsible for the toxicity of amygdalin (Abboud *et al.*, 2019; He *et al.*, 2020; Jaszczak-Wilke *et al.*, 2021). Apricot seeds possess hepatoprotective and anticancer activities that justify its traditional use, and its potential for the treatment of liver diseases including hepatocellular carcinoma (Ramadan *et al.*, 2020). Blaheta *et al.* (2016) and Saberi *et al.* (2022) concluded that the lethal dose for adults is 50 bitter almonds and (5-10) grains for children, the toxins are removed from

Cyanide (80%) in the liver. The presence of Rhodanase enzyme in the liver that works to stimulate the detoxification of cyanide because it gives the sulfur thiol group from the amino acids cysteine and methionine, which leads to the conversion of highly toxic cyanide to thiocyanate, which is less toxic and quickly excreted through the urine (Jaszczak-Wilke *et al.*, 2021).

Materials and Methods

The apricot kernel was obtained from the local Iraqi market in April (2020), it was washed, dried and preserved at room temperature.

Amygdalin extraction: The apricot kernels were ground with the mill and after the oil (Bolarinwa *et al.*, 2014) aqueous extraction according to different temperatures (37,100) C by taking 2 gm of ground almonds in a beaker and adding to it (50) ml of water and 0.25gm of citric acid and put the flask in a shaking water bath (37)^oC for (100) minutes, then filter the extract with Whatman No. 1 filter paper and take the filtrate for analysis by HPLC, the same method was followed with the extraction at 100 ° C but using a reflux condenser.

Anti-cancer efficacy: A laboratory experiment was conducted to study effect of amygdalin extracted from apricot kernels on breast cancer cell line MCF-7 in

comparison with hepatoma cell line (WRL-68) using Methyl Thiazolyl Tetrazolium (MTT) method (Rawa'a 2019). The cell vitality test was done according to (Freshney 2015)>

Cell viability (%) = (total viable cells(unstained))/ (total cells counted (stained& unstained)) × 100

Results and Discussion

Amygdalin extraction: The effect of heat on the concentration of extracted amygdalin was studied using HPLC (Table 1).

Table 1 : The effect of temperature on the concentration of amygdalin measured by HPLC technique.

Extraction temperature (C)	Amygdalin concentration (ppm)
37	20.3
100	68.2

The concentration of amygdalin at high temperature is higher than it is at low temperature (Table 1 and Figure 1) and this is consistent with what was mentioned (Bolarinwa *et al.*, 2014). The high concentration of amygdalin at high temperature (Figure 2) is due to the inactivation of hydrolytic enzymes which prevents the decomposition of amygdalin to hydrocyanic acid and benzaldehyde (Savic *et al.*, 2015). The addition of citric acid when extracting prevents the conversion of amygdalin to another compound (Neo-amygdalin) to maintain its anti-cancer activity (Boháčová *et al.*, 2019).

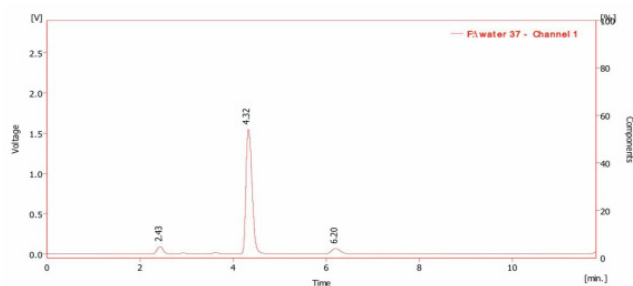


Fig. 1 : Concentration of amygdalin extracted at 37°C using HPLC

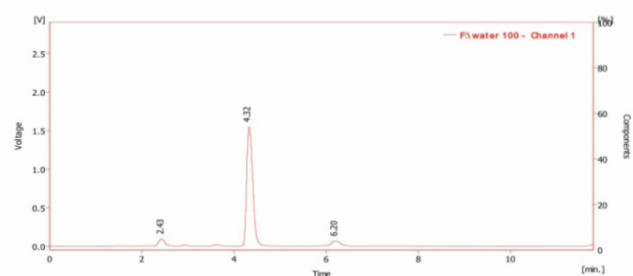


Fig. 2 : Concentration of amygdalin extracted at 100°C using HPLC

Anti-cancer efficacy: The rate of live cells for breast cancer MCF-7 was 34-82% at concentrations of 25-400 µg/ml and the concentration that inhibits 50% (IC50) is 82.74 µg/ml (Table 2, Figure 3). the rate of hepatocytes WRL-68 was 65-94% at the same concentrations 25-400 µg/ml and the IC50 value were 133.9 µg/ml. Amygdalin showed a greater effect on the toxicity of cancer cells than it is in healthy cells, due to the presence of rhodanese enzyme in the structure of normal cells, which converts cyanide to a less dangerous compound thiocyanate, as cancer cells lack this enzyme (Mosayyebi *et al.*, 2020; Okonji *et al.*, 2017). The cytotoxicity increases with the increase in the concentration of amygdalin, and this is consistent with what was indicated by (Arshi *et al.*, 2019) as it was shown that the inhibition of cancer cells increases with an increase in the concentration of amygdalin, explaining the role of amygdalin as an anti-carcinogen due to its effect in the proteins responsible for the apoptosis of cancer cells, where amygdalin increases the expression of Bax proteins and decreases the expression of Bcl-2 proteins, and also amygdalin increases the activation of caspase-3, which leads to the apoptosis process of cancer cells Jaszczak-Wilke *et al.*, 2021). Blaheta *et al.* (2016) showed that the cyano group present in the synthesis of amygdalin has anti-cancer activity by causing programmed death of cancer cells and inhibiting their growth in addition to reducing malignant tumors. In addition to the presence of amygdalin, the content of apricot kernels of oils and fatty acids such as Oleic, linoleic, and volatile oils such as benzaldehyde, phenols and flavonoids all represent a protective system against cancer diseases by causing changes in the permeability of the cancer cell membrane and morphological changes (Cassiem and de Koc 2019).

Table 2 : The viable cell count for amygdalin extracted from apricot kernels

Concentrations (mg/ml)	MCF-7		WRL-68	
	viable cell rate	SD	viable cell rate	SD
400	34.7	3.0	65.7	5.0
200	43.0	2.8	72.1	2.2
100	55.6	3.7	86.3	2.5
50	72.4	1.2	96.1	1.2
25	82.8	1.3	94.2	3.1

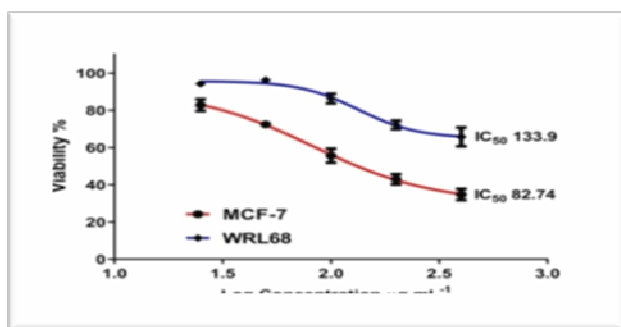


Fig. 3 : IC₅₀ of amygdalin extracted from apricot kernels against MCF-7 cells

Conclusion

stand on the active point of using apricot kernels to achieve amygdalin for the purpose of studying its anti-cancer activity using the MCF-7 breast cancer cell line, compared with the liver cell line (WRL-68), and it is clear that the effect of amygdaline was excellent on cancer cells, in future may be the chemical treatment could be replaced by using extracted apricot kernels.

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