Trop J Nat Prod Res, October 2020; 4(10):728-733 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

A Potent Anti-Ageing and Immunomodulatory Activity of Apricot Seed Standardized Extract and its Major Compound; Amygdalin

Amer Ramadan[1], Gehan Kamel[1], Aya A. Shokry[1]*, Riham A. El-Shiekh[2]

[1]*Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt*

ARTICLE INFO

Article history:
Received 05 September 2020
Revised 06 October 2020
Accepted 01 November 2020
Published online 02 November 2020

ABSTRACT

Ageing is an expected problem that affects the skin of all mammals and could be avoided by herbal protection. Therefore, our study was done to determine anti-ageing and immunomodulatory effect of apricot seed standardized extract and its major compound; amygdalin. The dried powder was extracted using 70% ethanol (3 × 1.5 L) by an Ultra-Turrax® T25 homogenizer, then the extract was investigated for its anti-ageing and immunomodulatory activity. *In-vitro* anti-cyclooxygenases (COX-1, COX-2), anti-collagenase, anti-elastase and immunomodulatory activities (lymphocyte proliferation and phagocytic activity) were done using standard methods. The results revealed that the apricot seed 70% ethanol extract showed a promising activity as anti-ageing by collagenase and elastase enzyme inhibition assays (IC_{50} was 44.29 ± 1.7 µg/mL and 37.23 ± 0.96 µg/mL, respectively). While, amygdalin had a potent activity as collagenase and elastase inhibitor (21.70 ± 0.92 µg/mL and 17.78 ± 1.3 µg/mL, respectively) which was better than the standard drug EGCG (IC_{50} was 24.7 ± 1.6 µg/mL and 18.2 ± 1.5 µg/mL, respectively). Immunostimulant results for apricot seed 70% ethanol extract and amygdalin increased in gradient concentration with valuable effects. Standardization of extract using HPLC analysis and amygdalin as marker was done where its content was 5.72 g/100 g extract. Vitamin E was estimated as 0.1052 mg/g dry powder of apricot seed and this revealed its nutritive importance. In conclusion, we recommended the use of apricot seed extract or amygdalin as therapeutic adjuvant in skin creams for prevention of skin ageing.

Keywords: Apricot seeds, Amygdalin, Immunostimulant, Skin wrinkles, Ageing.

Introduction

Apricot seeds are the kernel derived from *Prunus armeniaca* L. which belongs to family Rosaceae. Apricot is cultivated in South Africa, Asian countries, South Europe, and Australia.[1] The apricot seeds have been used to treat several skin infections, and to dehydrate, nourish, and lubricate the skin.[2] It was reported to have antiseptic, anti-oxidant, and anti-inflammatory remedy.[3] It is used traditionally as antitussive agent in asthma and bronchitis disorders. The oil of these seeds is commonly used in skin care products and in drug combinations as ingredients with medical importance.[4]

Amygdalin is a well-known glycoside containing cyanide and present in seeds of plants related to Rosacea family, reported as beneficial adjuvant in cancer treatment.[5]

Skin ageing is an unavoidable progression and all living organisms can suffer from it appearing in their skin. There are 2 types of skin ageing according to the cause, intrinsic and extrinsic ageing. Intrinsic skin ageing is due to changes in elasticity over time and known as age-dependent/chronological, where extrinsic is caused by the direct exposure to UV light and other destructive agents, so it is known as premature ageing/photoageing.[6,7] The collagenases and elastases enzymes cause degradation of collagen and elastin in our body and lead to the reduction of skin elasticity and development of wrinkles.[8]

Some medicinal herbs are commonly used in body and skin care products to prevent skin ageing and provide lightening of the skin,[9] as a result of the presence good amounts of carotenoids, phenolics, vitamins C and E.[10]

Medicinal plants with immunomodulation effects are preferred to synthetic ones.[11] The medicinal plants and their constituents can be used for modulation of immune functions. Many natural substances act on microorganisms not just by affecting the pathogen directly but they can act also by inducing natural and acquired immune response of the body.[12] Vitamin E especially α-tocopherols is a powerful antioxidant which is able to protect body cells from stress caused by free radicals by prevention of the oxidation of polyunsaturated fatty acids in cells. Vitamin E act as immunomodulator to enhance the immune functions of the host,[13,14] and so could be used as effective anti-wrinkles. Therefore, anti-ageing and immunomodulatory activities were tested in the study for apricot seed standardized extract and its major compound; amygdalin to support the search for new natural anti-wrinkles drug.

Materials and Methods

Chemicals, reagents and instruments

Collagenase enzyme from Clostridium histolyticum (ChC-EC.3.4.23.3), N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala substrate (FALGPA), epigallocatechin gallate (EGCG), porcine pancreatic elastase (PPE, Sigma, Type IV) enzyme, N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide were bought from Sigma Aldrich (St. Louis,

*Corresponding author. E mail: ayaabdelsalam333@cu.edu.eg.com
Tel: 01095977296

Citation: Ramadan A, Kamel G, Shokry AA, El-Shiekh RA. A Potent Anti-Ageing and Immunomodulatory Activity of Apricot Seed Standardized Extract and its Major Compound; Amygdalin. Trop J Nat Prod Res. 2020; 4(10):728-733. doi.org/10.26538/tjnpr/v4i10.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

MO, USA). Tricine buffer (50 mM, pH 7.5) was purchased from Biodiagnostic, Egypt. Phytohaemagglutinin (PHA, SIGMA) was bought from Sigma Aldrich (St. Louis, MO, USA). Zymosan from Saccharomyces cerevisiae was bought from Sigma Chemical Co. (St. Louis, MO). ELISA microplate reader spectrophotometer (ChroMate, 4300, FL, USA) was used.

Plant materials and extract preparation
Apricot seeds were bought from apricot farm during the season of apricot production (Summer 2019). The internal kernels were kept in bags tightly closed in the freezer for extract preparation. A voucher specimen (No. 1.10.2019) was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt and authenticated by Mrs Teresa Labib, Senior Taxonomist at Orman Botanic Garden.
The dried powder (500 g) was extracted using 70% ethanol (3×1.5 L) by an Ultra-Turrax® T25 homogenizer (Janke & Kunkel IKA-Lab., Staufen, Germany). The extract (32 g) was placed in the desiccator over anhydrous CaCl2 for further studies.

Isolation of amygdalin
The ethanol extract (1 g) was chromatographed on a silica gel RP-18 column (20 × 2.5 cm). Gradient elution was achieved by using 100% distilled water, then mixtures of methanol/water (5-60%), till 100% methanol. Fraction II, using methanol-water (1.5:8.5 v/v) as eluent was collected to afford compound 1 (Amygdalin, 15 mg).

Standardization of the extract
Instrumentation
HPLC analysis of the samples and standard was performed on Smart Line, Knauer, Germany, equipped with autosamplling injector, a quaternary pump, degasser G1322A and an ultraviolet detector (UV). The separation was carried out on an Eclipse XDB-C18 column (100 X 4.6 mm, particle size 5 µm) with a C18 guard column (Phenomenex, Torrance, CA) and operated at 30^{o}C.

Sample and standard preparation
To obtain the calibration curve, five concentrations (1, 10, 50, 100 and 150 µg/µL) of amygdalin were dissolved in methanol, covering concentration range required based on the level expected in the plant samples. An aliquot (20 µL) of each was injected in triplicates and corresponding areas of peak determined. The calibration curve was then designed by plotting mean peak areas against corresponding concentration. Methanol: Water 15:85 (v/v) was the mobile phase and flow rate 0.7 mL/min. The UV detector placed at 215 nm and data integration by claritychrom® software. The solutions of samples were filtered through Agilent Ecno 0.45 µm polytetrafluoroethylene (PTFE) membrane filter and degassed in an ultrasonic bath before use.

Phytochemical screening of the extract
Determination of total phenolics:
Total amount of phenolics in the extract was measured spectrophotometrically by Folin–Ciocalteu assay.[15]

Determination of total flavonoids:
The total flavonoids were measured by aluminum chloride colorimetric method.[15] It was expressed as µg rutin equivalent/g dry powder.

Determination of total antioxidant capacity
Total antioxidant capacity was determined by determination of DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity according to the previous method.[16] The standard was ascorbic acid.

Determination of nutritive values of the seeds
The calculated total carbohydrates, lipids and proteins were done according to the previously described methods.[17-19] Content of tocopherol or vitamin E was estimated according to the previous method.[20] The total energy in calories was calculated using the following equation: (Total lipids x 9) + (Total protein x 4) + (Total carbohydrates x 4).

In-vitro assays
COX-1/COX-2 inhibition
Apricot seed extract and amygdalin were evaluated for their ability to inhibit Cyclooxygenase (COX-1 and COX-2) using assay kit for COX (Item No.560131, CAYMAN chemicals, MI, USA). The selectivity index (S.I.) was calculated also as IC50 (COX-1)/IC50 (COX-2).[21]

Collagenase enzyme inhibition assay
The assay was done as previously described with some changes.[6] In brief, 20 µL of enzyme solution (0.8 U/mL) was added followed by 20 µL of test compounds (1000-7.81 µg/mL) and then 20 µL of Tricine buffer (50 mM, pH 7.5) in a 96-well microplate. After incubation period of 15 min at 37°C, 40 µL of the substrate (2 mM) was added. The absorbance was determined at 335 nm directly after addition of the substrate and kept for 20 minutes using a microplate reader (Tecan, USA). Water was used as negative control and EGCG as standard. The IC_{50} value, known as the concentration of sample to inhibit 50% of collagenase was determined.

Elastase enzyme inhibition method
The elastase inhibition activity was determined as previously described with minor changes.[22] In brief, 25 µL of 0.1 M HEPES buffer (pH 7.5), tested samples (1000-7.81 µg/mL) and elastase enzyme (1 µg/mL) were added into 96-well plates. After 20 min incubation period, 100 µL of the substrate N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (1 mM) was added followed by another incubation period of 40 min at 25°C. The absorbance was measured at 405 nm. EGCG was used as a standard.

Immunomodulation assay
Lymphocyte proliferation activity using MTT reduction assay
The proliferation activity was measured by determination of the mitochondrial activity using the MTT reduction assay.[23] There was a direct relationship between the proliferation of cells and the MTT reduction values. Viable lymphocyte number was calculated using haemocytometer by equation and lymphocyte proliferative response can be estimated by stimulation of lymphocyte with phytohaemagglutinin at concentration of 15 µg/mL and cultured with 10% fetal calf serum at 37°C and 5% CO_2 for 72 h. After that, a solution of MTT in PBS (5 mg/mL) was added to each well culture then incubated for 5 h. When MTT is reduced, it is converted to blue formazan crystals during incubation. After incubation, these crystals were dissolved by adding 10% SDS in 0.01M HCL during an additional incubation period of 16 h. The absorbance was read at 590 nm by ELISA reader.

Phagocytic activity by measuring nitric oxide concentration
The buffy coats of healthy donors were used for isolation of monocytes, incubated at 37^{o}C for 2 h, after that the non-adhered cells were discarded. The monocytes are transformed into macrophages after their culture in the presence of 10% bovine serum and last for 3-5 days. Zymosan from Saccharomyces cerevisiae was washed with sterilized phosphate buffer saline, then coating with complement through process of opsonization by incubation with serum for 1 h at 37^{o}C, then centrifuged and resuspended in sterilized PBS. The cells were incubated with zymosan particles for 1 h, 2 h and overnight, at each time the supernatant over macrophage was collected and nitric acid concentration was measured in it.[24] Nitric oxide levels were assessed as the previously described method.[25]

Statistical procedures
The analysis was done using SPSS (2020). All results were expressed as means ± SD. One-way ANOVA and Duncan test were used. $P< 0.05$ were regarded as statistically significant.

Results and Discussion

Isolation of amygdalin
Plant metabolites have been reported for their different activities such as antioxidant, anti-inflammatory and anti-ageing. Co-chromatography with authentic sample revealed the identification of compound 1 as

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Amygdalin (R_f = 0.38 in methylene chloride/methanol (9.4:0.6 v/v) (Figure 1). The amount of the major constituent; amygdalin of Apricot seeds was calculated from the performed calibration curve using the peak area of the extracted UV. HPLC analysis showed that the amount of amygdalin was measured to be 5.72 g/100 g extract according to the chromatogram (Figure 2).

Phytochemical screening of t0he extract

It was done for the crude ethanol extract of apricot seeds. Total phenols, flavonoids, carbohydrates, lipids, protein, tocopherols (vitamin E), fibers, energy was calculated and reported in Table 1. The medicinal herbs able to protect all skin and cell structures from damage caused by oxidative stress and prevent oxidation by free radicals which increase with age.[26] DPPH method is a common potent test which measure the ability of plant extracts as antioxidant.[27,28] The total antioxidant capacity of our apricot seed extract was remarkable, coupled with its effect as anti-ageing confirmed by elastase and collagenase inhibition activities. The antioxidant activity was further confirmed by the total phenolics and flavonoids in the extract. Presence of these substances in considerable amounts explain their effect as anti-ageing and immunomodulatory agents. It is worth to highlight the vitamin E content which was 0.1052 mg/g dry weight powder. The nutritive value of the extract also confirmed high value of vitamin E which plays a good role as antioxidant.

In-vitro assays

COX-1/COX-2 Inhibition

The results in Table 2 show the concentration causing 50% enzyme inhibition (IC_{50}) in addition to the selectivity index (SI). Amygdalin showed a potent anti-inflammatory activity with IC_{50} of 8.7 µM and 0.064 µM for COX-1 and COX-2, respectively while the extract was 10.4 µM and 0.33 µM for COX-1 and COX-2, respectively in comparison with standard which was about 15.1 µM and 0.064 µM for COX-1 and COX-2, respectively. The anti-inflammatory activity of ethanol apricot kernels extract and amygdalin was remarkable and indicated that amygdalin itself is a potent anti-inflammatory agent.

Collagenase and elastase enzymes inhibition assay

Wrinkles or skin ageing is a common problem which occurs due to different factors such as the direct exposure to UV irradiation, genetic effect due to decrease in the ability of the body cells to interact with reactive oxygen species (ROS) or occur with time. Normally, the skin contains collagen and elastin which are formed and degraded continuously to keep the natural structure of the skin and protect it from damage.[29] Skin and body care products contain natural ingredients which contain anti-elastase, anti-collagenase and antioxidant activities, so can be used to decrease skin ageing. These natural ingredients include several phytochemicals such as saponins, terpenoids, vitamins, carotenoids, alkaloids, polyphenols, and tannins. These compounds have potent antioxidant activities, which can be used in the treatment of multiple ailments such as the ageing process by decreasing the amount of ROS. Inhibition of collagenase and elastase enzymes is required to prevent skin problems.[30] So, the extract and amygdalin were assessed as elastase and collagenase enzymes inhibitors.[31] The extract and amygdalin ability to inhibit collagenase and elastase enzymes was determined as shown in Table 3. EGCG decreased enzymatic effect in a concentration-dependent manner. Amygdalin showed a potent activity against collagenase and elastase with IC_{50} = 21.70 ± 0.92 µg/mL and 17.78 ± 1.3 µg/mL, respectively compared to EGCG (24.7 ± 1.6 µg/mL and 18.2 ± 1.5 µg/mL, respectively). Our results revealed that the apricot seed 70% ethanol extract possess a very promising activity as anti-ageing. The ethanol apricot seed extract and amygdalin showed significant effect as anti-elastase that was comparable to EGCG. Most of the phenolics and flavonoids are able to inhibit elastase activity according to their concentration.[32,33] The major chemical constituents of apricot seeds previously measured are phenolics, flavonoids, carotenoids and amygdalin. From all these results, we can conclude the amygdalin has more powerful anti-ageing effect than ethanol apricot seeds extract.

Lymphocyte proliferation activity using MTT reduction assay and Phagocytic activity by measuring nitric oxide concentration

Natural substances from medicinal plants are able to enhance the innate immunity against different infections and can be used as immunostimulant.[34] Some medicinal plants have shown potent immune response in previous studies such as garlic,[35] milk thistles,[36] ginseng,[37] and Echinacea.[38] Immunomodulation through medicinal plants can serve as an alternative to using chemical drugs for different diseases, especially in case of impaired immune response, and the host defense mechanism must be activated or in case of autoimmune disorders.[39] The immunomodulatory effect of amygdalin and ethanol apricot seed extract were determined by lymphocyte proliferation assay and macrophage stimulation. Macrophages are one of the important cells that stimulate cell-mediated and antibody mediated immune responses.[40,41] Nitric oxide (NO) is well-known as an important effector molecule in the diminishing of microorganisms by macrophages.[42] Lymphocyte transformation assay is a simple, rapid and uncomplicated technique used to assess immune responses. This assay illustrates the mitogenic effect of the tested substance on T-lymphocyte proliferation. Immunosuppression is indicated by anti-proliferative effect of T-lymphocyte while immunostimulant is indicated by increase in T-lymphocyte proliferation.[43] The results for amygdalin and ethanol apricot seeds extract showed immunostimulant effect in a concentration-gradient manner as shown in Table 4. Treatment of lymphocytes with the ethanol apricot seed extract and amygdalin revealed a potent immunostimulatory effect by increasing lymphocyte proliferation percent by increasing their concentration.

Treatment with amygdalin and ethanol apricot seeds extract on the macrophages revealed an increase in the nitrites level produced by these cells in different levels according to the concentration gradient Table 5. NO production in the negative control group increased up to 10.4 µM. The ethanol apricot seed extract and amygdalin boosted the macrophages' phagocytic activity enthused by zymosan in a dose-dependent manner. Vitamin E is a powerful antioxidant natural substance and immunomodulator which stimulate the host immune response. It stimulates B cell and macrophage functions. Vitamin E is used as dietary supplement to suppress pro-inflammatory cytokines and chemokines.[44]

Table 1: Results of total phenols, total flavonoids, total anti-oxidant capacity and nutritive values of 70% ethanol apricot seeds extract

Assay	Result
Total phenols	12.75 µg gallic acid equivalent/g dry powder
Flavonoid content	16.96 µg rutin equivalent /g dry powder
Total antioxidant capacity (DPPH)	42.16 µg ascorbic acid equivalent /g dry powder
Total energy	561.25 Calorie
Total carbohydrates	0.4946 gram/gram dried powder
Total lipids	0.3321 gram/gram dried powder
Total protein	0.1613 gram/gram dried powder
Fiber	0.1952 gram/gram dried powder
Vitamin E	0.1052 milligram/gram dried powder

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: *In vitro* COX-1 and COX-2 (IC_{50}, µg/ml) for both 70 % ethanolic apricot seeds extract and amygdalin

	(IC_{50}, µg/mL)		SI
	COX-1	COX-2	
70% Ethanol Extract	10.40 ± 0.39^{b}	0.33 ± 0.03^{b}	31.51
Amygdalin	8.70 ± 0.34^{a}	0.064 ± 0.04^{a}	135.94
Celecoxib	15.10 ± 0.21^{a}	0.049 ± 0.02^{a}	308.16

Table 3: *In vitro* collagenase and elastase (IC_{50}, µg/mL) of both 70% ethanolic extract of apricot seeds and amygdalin

	(IC_{50}, µg/mL)	
	Collagenase	Elastase
70% Ethanol Extract	44.29 ± 1.70^{b}	37.23 ± 0.96^{b}
Amygdalin	21.70 ± 0.92^{a}	17.78 ± 1.30^{a}
EGCG	24.70 ± 1.60^{a}	18.20 ± 1.50^{a}

Data are represented as mean ± SD. The letters (a & b) are considered significant at $P<0.05$ in comparison with standard.

Table 4: Effect of different concentrations of 70% ethanol apricot seeds extract and amygdalin on peripheral blood lymphocytes proliferation

	Absolute lymphocytes count ($x10^3$ cells/µL)	
Conc. (µg /mL)	**70% Ethanol Extract**	**Amygdalin**
0 (control)	0.96 ± 0.21^{a}	0.96 ± 0.21^{a}
1000	4.32 ± 0.36^{j}	4.25 ± 0.44^{j}
500	4.09 ± 0.40^{i}	3.76 ± 0.23^{i}
250	4.03 ± 0.34^{h}	3.01 ± 0.54^{h}
125	3.91 ± 0.37^{g}	2.71 ± 0.58^{g}
62.5	3.65 ± 0.45^{f}	2.46 ± 0.32^{f}
31.25	3.42 ± 0.47^{e}	2.33 ± 0.34^{e}
15.6	3.31 ± 0.18^{d}	2.23 ± 0.21^{d}
7.5	2.85 ± 0.16^{c}	2.12 ± 0.64^{c}
3.75	2.57 ± 0.15^{b}	1.6 ± 0.42^{b}

Data are represented as mean ± SD. The letters (a-j) are considered significant at $P < 0.05$ in comparison with control.

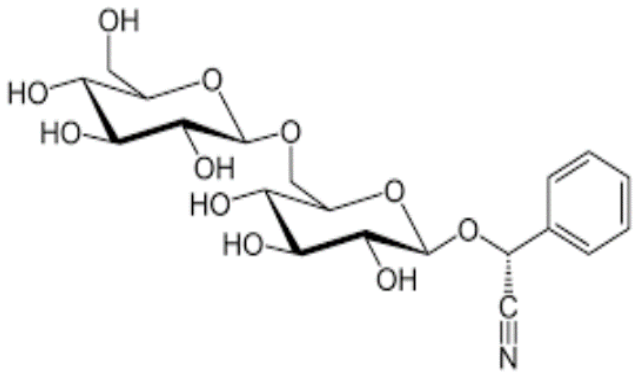

Figure 1: Chemical structure of amygdalin

Table 5: Effect of different concentrations for both 70% ethanol apricot seeds extract and amygdalin on nitric oxide concentration produced by macrophages

Conc. (µg /mL)	Nitric oxide concentration (µg/mL)	
	70% Ethanol Extract	Amygdalin
0 (control)	10.4 ± 1.14^{a}	10.4 ± 1.14^{a}
1000	23.13 ± 1.14^{j}	16.77 ± 1.2^{j}
500	17.11 ± 0.47^{i}	16.53 ± 1.5^{i}
250	17.13 ± 0.8^{h}	15.56 ± 1.1^{h}
125	16.5 ± 1.5^{g}	15.03 ± 0.9^{g}
62.5	15.13 ± 1.7^{f}	13.49 ± 0.7^{f}
31.25	15.09 ± 1.32^{e}	10.23 ± 1.7^{e}
15.6	14.17 ± 1.9^{d}	9.97 ± 0.5^{d}
7.5	13.27 ± 0.8^{c}	9.57 ± 1.3^{c}
3.75	12.30 ± 0.57^{b}	8.88 ± 1.1^{b}

Data are represented as mean ± SD. The letters (a-j) are considered significant at $P<0.05$ in comparison with control.

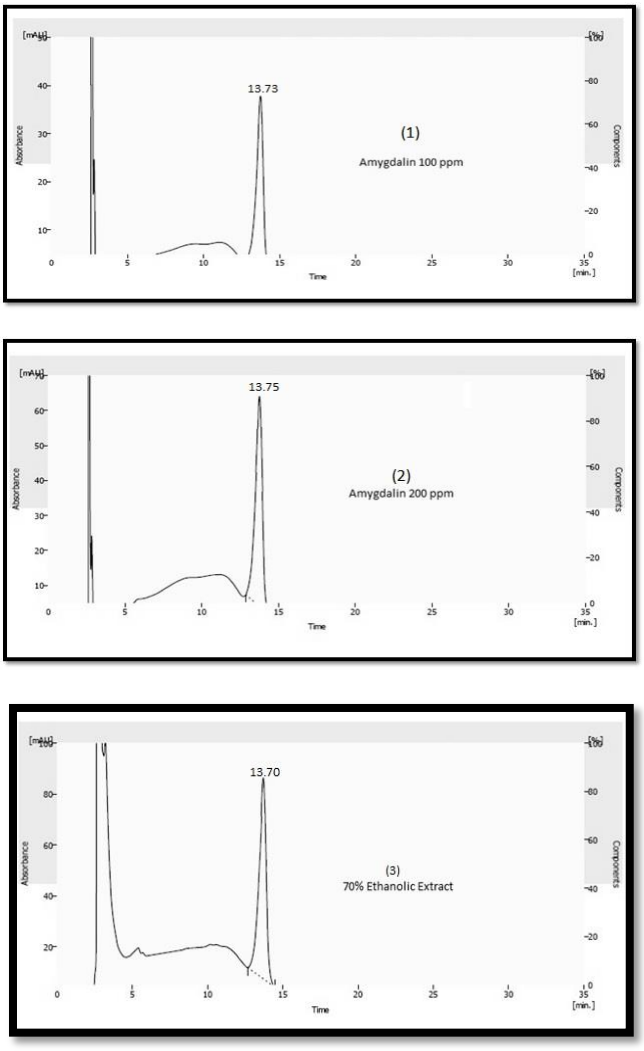

Figure 2: HPLC Chromatograms of amygdalin (1,2) and 70% ethanol apricot seeds extract (3).

Conclusion

Apricot seeds are rich source of phenolics, flavonoids, carotenoids and amygdalin. So, they are a promising candidate for the treatment of skin diseases such as skin wrinkles. Apricot seeds ethanol extract had a potent anti-COXs, anti-collagenase and anti-elastase activities as well as immunostimulant potential. So, we recommended the use of their extract in cosmetic skin creams as anti-wrinkle and immunostimulant component.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Kim CS, Jo K, Lee IS, Kim J. Topical application of apricot kernel extract improves dry eye symptoms in a unilateral exorbital lacrimal gland excision mouse. Nutrients. 2016; 8(11):750-760.
   Ramadan A, Kamel G, Awad NE, Shokry A. Phytochemical screening, acute toxicity, analgesic and anti-inflammatory effects of apricot seeds ethanolic extracts. J Appl Vet Sci. 2018; 3(1):26-33.
2. Hyun S, Kim J, Park B, Jo K, Lee TG, Kim JS, Kim CS. Apricot kernel extract and amygdalin inhibit urban particulate matter-induced keratoconjunctivitis sicca. Mol. 2019; 24(3):650-662.
3. Ramadan A, Kamel G, Awad NE, Shokry AA, Fayed HM. The pharmacological effect of apricot seeds extracts and amygdalin in experimentally induced liver damage and hepatocellular carcinoma. J Herbmed Pharmacol. 2020; 9(4):400-407.
4. Cassiem W, De Kock M. The anti-proliferative effect of apricot and peach kernel extracts on human colon cancer cells in vitro. BMC Compl Altern Med. 2019; 19:32-44.
5. Thring TS, Hili P, Naughton DP. Anticollagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Compl Altern Med. 2009; 9:27-38.
6. Mukherjee PK, Maity N, Nema NK, Sarkarm BK. Bioactive compounds from natural resources against skin ageing. Phytomedicine. 2011; 19:64-73.
7. Ndlovu G, Fouche G, Tselanyane M, Cordier W, Steenkamp V. In vitro determination of the anti-ageing potential of four southern African medicinal plants. BMC Compl Altern Med. 2013; 13:304-311.
8. Hsu MF and Chiang BH. Stimulating effects of Bacillus subtilis natto-fermented Radix astragali on hyaluronic acid production in human skin cells. J Ethnopharmacol. 2009; 125:474-481.
9. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017; 18(1):96-128.
10. Mukherjee PK, Nema NK, Bhadra S, Mukherjee D, Braga FC, Matsabisa M. Immunomodulatory leads from medicinal plants. Indian J Tradit Know. 2014; 13(2):235-256.
11. Shukla S, Bajpai VK, Kim M. Plants as potential sources of natural immunomodulators. Rev in Environ Sci Biotechnol. 2014; 13(1):17-33.
12. Pekmezci D. Vitamin E and immunity. Vitam Horm. 2011; 86,179-215.
13. Salimian J, Arefpour MA, Riazipour M, Poursasan N. Immunomodulatory effects of selenium and vitamin E on alterations in T-lymphocyte subsets induced by T-2 toxin. Immunopharmacol Immunotoxicol. 2014; 36(4):275-281.
14. Baba SA and Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci. 2015; 9(4):449 - 454.
15. Villano D, Fernandez-Pachon MS, Moya ML, Troncoso AM, Garcıa-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2007; 71:230-235.
16. Kostas TE, Wilkinson JS, White AD, Cook JD. Optimization of a total acid hydrolysis-based protocol for the quantification of carbohydrates in macro algae. J Algal Biomass Util. 2016; 7(1):21-36.
17. AOAC. Official method of analysis of the association of official analytical chemist. 15th ed., Washington, D.C., USA, 1990.
18. AOAC. Method of analysis, Association of Official Agriculture Chemists. 16th ed., Washington, D.C., USA, 1995.
19. Emmerie A and Engel C. Colorimetric Determination of Tocopherol (Vitamin E): II Adsorption Experiments. Rec Trav Chim. 1939; 58(4):283-289.
20. George A, Chinnappan S, Chintamaneni M, Kotak CV, Choudhary Y, Kueper T, Radhakrishnan AK. Anti-inflammatory effects of *Polygonum minus* (Huds) extract (LineminusTM) in in-vitro enzyme assays and carrageenan induced paw edema. BMC Compl Altern Med. 2014; 14:1–7.
21. Kraunsoe JAE, Claridge TDW, Lowe G. Inhibition of human leukocyte and porcine pancreatic elastase by homologues of bovine pancreatic trypsin inhibitor. Biochem. 1996; 35:9090–9096.
22. Rai-Elbalhaa G, Pellerin JL, Bodin G, Abdullah HA, Hiron, H. Lymphocytic transformation assay of sheep peripheral blood lymphocytes. A new rapid and easy to read technique. Microbiol Inf Dis. 1985; 8:311-318.
23. Municio C, Alvarez Y, Montero O, Hugo E, Rodríguez M, Domingo E, Alonso S, Fernadez N, Crespo MS. The response of human macrophages to β-Glucans depends on the inflammatory milieu. PloS One. 2013;8(4):e62016.
24. Rajaraman V, Nonnecke BJ, Franklin ST, Hamell DC, Horst RL. Effect of vitamin A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. J Dairy Sci. 1998; 81:3278-3285.
25. Fu L, Xu BT, Gan RY, Zhang Y, Xu XR, Xia EQ, Li HB. Total phenolic contents and antioxidant capacities of herbal and tea infusions, Int J Mol Sci. 2011; 12(4):2112–2124.
26. Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. Antioxidant activity in some selected Indian medicinal plants. Afr J Biotechnol. 2008; 7(12):1826–1828.
27. Maizura M, Aminah A, Wan Aida WM. Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. Int Food Res J. 2011; 18:529- 534.
28. Takeuchi H, Gomi T, Shishido M, Watanabe H, Suenobu N. Neutrophil elastase contributes to extracellular matrix damage induced by chronic low-dose UV irradiation in a hairless mouse photoageing model. J Dermatol Sci. 2010; 60:151–158.
29. Kim T, Kim HJ, Cho SK, Kang WY, Baek H, Jeon HY, Kim B, Kim D. *Nelumbo nucifera* extracts as whitening and anti-wrinkle cosmetic agent. Korean J Chem Eng. 2011; 28:424–427.
30. Kashif M, Akhtar N, Mustafa R. An overview of dermatological and cosmeceutical benefits of diospyros kaki and its phytoconstituents. Revista Brasileira De Farmacognosia. 2017; 27:650–662.
31. Wittenauer J, Mackle S, Sussmann D, Schweiggert-Weisz U, Carle R. Inhibitory effects of polyphenols from grapepomace extract on collagenase and elastase activity. Fitoterapia. 2015; 101:179–187.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

32. Lee SM, Song YH, Uddin Z, Ban YJ, Park KH. Prenylated flavonoids from epimedium koreanum nakai and their human neutrophil elastase inhibitory effects. Rec Nat Prod. 2017; 11(6):514-520.

33. Singh N, Tailang M, Mehta SC. A review on herbal plants as immunomodulators. IJPSR. 2016; 7(9):3602-3610.