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PHYTOCHEMICAL SCREENING, ACUTE TOXICITY , ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF APRICOT SEEDS ETHANOLIC EXTRACTS

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ABSTRACT

The aim of present study was to investigate the phytochemical screening, acute toxicity and some pharmacological activities of ethanolic extract 70 % and 99.9% of apricot seeds. The phytochemical screening was done for determination of total phenolic compounds by Folin–Ciocalteu method, total flavonoids by aluminum chloride colorimetric method and total carotenoids by colorimetric method. The acute toxicity was done for determination of LD_{50} in mice by oral administration of upgraded doses of the extracts. The anti-inflammatory activity was done *in vivo* by formalin-induced paw edema in rats. The analgesic activity was carried out in mice by writhing test and hot plate method. Phytochemical screening revealed that the amount of total phenolic compounds was 179.4 and 191.2 µg gallic acid equivalent / g dry extract and the amount of total flavonoids was 226.18 and 509.34 µg rutin equivalent / g dry extract and the amount of total carotenoids was 0.145 and 0.156 mg/g dry extract for 70 % and 99.9% ethanolic extract, respectively. The acute toxicity revealed that both extracts had no toxic symptoms in rats and no mortalities appear by upgraded doses of 1 g to 10 g / kg b.wt. The 70% and 99.9% ethanolic extracts exhibited significant analgesic and anti-inflammatory activites in a dose of 100 mg/kg b.wt. of both extracts. These results suggest that apricot seed extracts contain significant level of safe non-toxic phytochemical substances that have significant analgesic and anti-inflammatory activities.

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INTRODUCTION

Apricot *(Prunus armeniaca* L.) is classified under the *Prunus* species of Rosaceae family of the Rosales group. Apricot has an important role in human diet, and can be used as fresh, dried or processed fruit **(Gezer *et al.*, 2011).** Apricots are processed in large amounts and huge consumption of apricot fruit produced by large amount of seeds **(Gornas *et al.*, 2015).** The apricot has been used in folk medicine for the treatment of different diseases. The bark of apricot tree used in the form of decoction with soothing effect can be applied on irritated skin. Other uses for apricots in folk medicine include treatment of hemorrhages, infertility, eye inflammation, and spasm **(Chevallier, 1996).** The different parts of the plant are used as food additives **(Asma *et al.*, 2007).**

Armeniacae semen is the seed of the apricot, the inner part of the apricot **(Hwang *et al.*, 2008).** Apricot seeds are usually roasted and mixed with coriander seeds and salt used as Dokka to be eaten as part of Egyptian folk diet **(Abdel-rahman 2011).** It is known that apricot seeds are exported to European countries to be used in medicine, cosmetic and oil production **(Gezer *et al.*, 2002).** Apricot seeds are added to bakery products (as whole or ground) in retail bakeries and also consumed as appetizers **(Durmaz and Alpaslan 2007).** *Armeniacae semen* is known to have many therapeutic effects such as relieving fever, respiratory symptoms such as wheeze, asthma, emphysema and bronchitis, stopping cough, quenching thirst. In traditional oriental medicine, *Armeniacae semen* has been used for the treatment of asthma, bronchitis, emphysema, constipation, nausea, leprosy,

and leukoderma **(Hwang *et al.*, 2003 and Bensky *et al.*, 2004**).

Apricot kernel paste can heal vaginal infections. The kernel oil has been used in cosmetics and as a pharmaceutical agent (laxative and expectorant). **(Chevallier, 1996).** It has been used for treating skin diseases such as furuncle, acne vulgaris and dandruff, as well as constipation **(Ju *et al.*, 2004).** *Armeniacae semen* is divided into the outer husk and an inner part that contains glycoside, amygdaline, starch, and fatty acids **(Chang *et al.*, 2005).** Some fruit seeds such as cherry, apricot, citrus and apple can be used as good source of oils. These seed oils are used for several purposes such as blending with highly saturated edible oils which are expensive to provide new oils with modified nutritional values as ingredients in paint and varnish formulations, surface coatings and oleo-chemicals, and as oils for cosmetic purposes **(Helmy 1990).**

There is no general utilization of apricot seeds, usually the amount that is collected goes into the adulteration of both almond kernels and their oil **(Aggarwal *et al.*. 1974).** The aim of the present study was to explore and confirm the phytochemical screening of apricot seed extracts followed by determination of toxicological effect of these extracts on rats and then determination of some pharmacological activities of these extracts *in vivo.*

MATERIALS AND METHODS

Seeds

Apricot seeds were collected from Edfina factory from Alexandria during the season of apricot production.

Seeds preparation

The seeds were washed, dried and then the kernels were splitted from the shell. After that, the internal kernels were kept in bags tightly closed in the freezer for preparation of the extract.

Extract preparation

Ethanolic extract 99.9%

The kernels were soaked in 99.9 % ethanol solution at room temperature. The kernels were grinded in a mortar using pestle and then exposed to boiling for 100 min, at 55 ^{0}C .Extract was then filtered through a layer gauze. The solvent was removed using rotatory evaporator apparatus attached with vacuum pump and temperature 40-50 ^{0}C. The yield was chilled in a refrigerator until use.

Ethanolic extract 70 %

Another type of extract was obtained by using 70 % ethanol solution and 6 % citric acid. The seeds were put in blender with water and citric acid till complete grinding and then the seeds put in flask containing 70 % ethanol solution for boiling at 55 ^{0}C for 100 min. Extract was then filtered. The solvent was removed using rotatory evaporator apparatus attached with vacuum pump and temperature was 40- 50^{0}C. The yield was chilled in a refrigerator until use.

Animals

Mature mice of both sexes and weighing (20-25 g) were used for studying the acute toxicity, LD50 and analgesic activity. Mature albino rats of both sexes and weighing (200-250 g) were used to reveal the anti-inflammatory activity. Standard rat pellets and tap water were supplied *ad libitum.*

Chemicals

All chemicals were purchased from Sigma-USA, Aldrich. Solvents were purchased from El Nasr Pharmaceutical chemicals Co., Egypt.

Phytochemical screening

Determination of total phenolic content

The total phenolic content of the extracts was determined spectrophotometrically by Folin–Ciocalteu method as described by **Baba & Malik (2015)**. Gallic acid was used as the reference standard and the results were expressed as µg of gallic acid equivalent per gram extract.

Determination of total carotenoid content:

The total carotenoid content of the extracts was determined spectrophotometrically by the method described by **Schertz (1923).** The extracts were reconstituted in 1 to 10 ml of acetone and sonicated for 2 min. visible spectra (340 to 700 nm , 1 nm interval)were collected using a 1 ml quartz cuvette in a UV/visible spectrophotometer.

Determination of total flavonoid content

The total flavonoid content of crude extracts was determined by aluminum chloride colorimetric method as described by **Baba & Malik (2015).** Rutin was used as a reference standard and the results were expressed as µg Rutin equivalent / gram of the extract.

Determination of acute toxicity

A pilot experiment was performed to determine LD_{50} of 70% and 99.9 % ethanolic extracts of apricot seeds. The LD_{50} was determined using method described by **Kerber (1941).** For this purpose 5 groups of five mice each weighing 20-25 g were administrated orally in upgrading doses ranging from 1000 to 10000 mg/kg b.wt. Another group was left as control and given vehicle only. The toxic symptoms and mortality rate in each group was recorded 72 hours post administration. The LD_{50} of the tested extract was calculated according to the formula using method described by **Kerber (1941).**

Determination of anti-inflammatory activity

According to the method described by **Domenjoz *et al.*, (1995).** Thirty rats of both sexes weighing 200-250 g were used. Rats were divided into five equal groups of five rats each. Rats of the first group were left as negative control (no inflammation, no treatment). Rats of the second group were left as positive control with induced inflammation only. The animals of the third group were orally administered indomethacin as standard drug in a dose of 10 mg/kg b.wt. **(Akindele & Adeyemi 2007).** Rats of the fourth and fifth groups were orally administered 70 % and 99.9 % ethanolic extracts at a dose of 100 mg/kg b.wt. Thirty minutes after drug or tested compound administration, 0.1 ml of 2.5 % formalin solution in normal saline was injected subcutaneously in the right hand paw of all animals for induction of edema. The thickness of each rat paw was measured in mm by Vernier caliber after 1, 2, 3, 4 and 5 hours post administration.

Determination of analgesic activity

A) Acetic acid induced writhing test

This method was used to evaluate the possible peripheral effects of ethanloic extracts of apricot seeds. This experiment was carried as described by **Okun *et al.*, (1963).** Thirty mice of both sexes weighing 20-25 g were selected and divided into 4 groups. Mice of the first group were kept as control non-treated, those of the second group were orally administered diclofenac sodium in a dose of 25 mg/kg b.wt. as a standard group **(Ahmed *et al.*, 2004).** Mice of the third and fourth groups were orally administered 70 % and 99.9 % ethanolic extract in a dose of 100 mg/kg b.wt. After 30 minutes, each mouse was intraperitoneally injected with 0.25 ml of 0.7 % glacial acetic acid in distilled water and the mice were then placed in transparent boxes for observation of writhing (arching of back, turning of trunk (twist), extension of hind limbs, contraction of abdominal musculature, torsion to one side so that the belly of the mouse touch the floor. Number of writhes of each animal in all groups was recorded within 30 min. and the analgesic potency of the tested extracts was determined as protection % against writhing according to the following formula:

$$\% \text{ of protection} = \frac{\text{control mean} - \text{treated mean}}{\text{control mean}} \times 100$$

B) Eddy's Hot plate test

The hot plate test was employed for assessment of possible centrally mediated analgesic effects of ethanolic extracts of apricot seeds. The experiment was carried out as described by **Janssen and Jageneau (1957)** and modified by **Jacob and Bosovski (1961)** using hot plate apparatus, thermostatically controlled at 55 ± 0.5 ^{0}C. Thirty mice were divided into 4 groups, 5 animals each. Reaction time (licking of paws or jumping off the hot plate) was measured prior to extract (min 0) and after the drug treatment. Mice of the first group were kept as control non-treated group. Mice of the second group were orally administered ibuprofen at a dose of 50 mg /kg b.wt. as a standard drug. Mice of the third and fourth groups were orally administered 70 % and 99.9 % ethanolic extract in a dose of 100 mg/kg b.wt., respectively. The reaction time was measured at 30 min. and repeated at 60, 90 and 120 min. post administration.

Statistical analysis

Data were presented as means ± standard errors. Data were analyzed using commercial software statistical package for social science (SPSS version©16). The significance level at P value ≤ 0.05 is considered significant.

RESULTS

Extract preparation

Ethanolic extract 99.9%

Each 250 gram seeds yielded 70 ± 2 gm of dried extract after complete evaporation of ethanol.

Ethanolic extract 70%

Each 250 gram seeds yielded 170 ± 2 gm of dried extract after complete evaporation of ethanol.

Determination of total phenols, total flavonoids, and total carotenoids in both 70 % and 99.9 % ethanolic extracts

The amount of total phenolic compounds, total flavonoids and total carotenoids are listed in Table (1).

Table 1: The amount of total phenols, total flavonoids, and total carotenoids in both 70 % and 99.9 % ethanolic extracts.

	70 % Ethanolic Extract	99.9 % Ethanolic Extract
Total phenols (µg gallic acid equivalent / g dry extract)	179.4	191.2
Total flavonoids (µg rutin equivalent / g dry extract)	226.18	509.34
Total carotenoids (mg/g)	0.145	0.156
β- carotene (mg/g)	0.01	0.01

Determination of acute toxicity

After oral administration of both 70 %, 99.9% ethanolic extract of apricot seeds in rats in a dose of 1, 3,5,10 g/kg b.wt. No deaths were observed during the 72 hr period at the tested doses. At these doses, the animals showed no toxic symptoms. The median lethal dose (LD50) was determined to be higher than highest tested dose in both samples. These results indicate the safety of the tested samples.

Determination of anti-inflammatory activity

The anti-inflammatory effect of the 70% and 99.9 % ethanolic extracts of apricot seeds was studied using formalin induced paw edema in rats and data were compared with that of control in Table (2). Oral administration of 70% and 99.9 % ethanolic extracts of apricot seeds in a dose of 100 mg/kg b.wt. induced a significant decrease in inflamed rat paw thickness when compared with control non treated group for 4 hours.

Determination of analgesic activity

A) Writhing test

The peripheral anti-nociceptive activity of the 70% and 99.9 % ethanolic extracts of apricot seeds was studied using acetic acid induced writhing technique in mice and the results were recorded in Table (3).

B) Hot plate test

Results of central anti-nociceptive activity of the 70% and 99.9 % ethanolic extracts of apricot seeds are recorded in Table (4).

DISCUSSION

This study investigated the phytochemical and toxicological and some pharmacological activity of Apricot seed extracts. The phytochemical screening included determination of total phenols, total flavonoids, total carotenoids and β-carotene in both 70 % and 99.9 % of armeniacae semen ethanolic extracts. The total phenolic content of the ethanolic extracts, calculated from the calibration curve($R2$= 0.9978), was 179.4 , 191.2 µg gallic acid equivalents/g dry extract in both70 % and 99.9 % ethanolic extracts respectively, the total flavonoid contents ($R2$= 0.9959) were 226.18 and 509.34 µg rutin equivalents/g dry extract in both 70 % and 99.9 % ethanolic extracts, respectively. The total carotenoids were 0.145 and 0.156 mg/g dry extract in both70 % and 99.9 % ethanolic extracts. respectively, and β – carotene content was 0.01 mg/g dry extract in both70 % and 99.9 % ethanolic extracts respectively as shown in (Table 1). Phenolic compounds have redox properties, so they can act as antioxidants and act as free radical terminators **(Soobrattee *et al.*, 2005 & Shahadi *et al.*, 1992).** They have free radical scavenging ability which is facilitated by their hydroxyl groups, the total phenolic concentration could be used as an indicator for rapid screening of total antioxidant capacity of the extract. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids act as effective antioxidant *in vitro* and *in vivo* **(Geetha *et al.*, 2003 & Shimoi *et al.*, 1996).**

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health should be considered. The mechanisms of action of flavonoids as antioxidants are through scavenging or chelating process **(Kessler *et al.*, 2003 & Cook and Samman 1996).** Carotenoids are plant pigments that function as antioxidants, hormone precursors, colorants and essential components of the photosynthetic apparatus. The presence of carotenoids in food crops are essential components for human diets **(Howitt & Pogson 1992).** β -carotene is a precursor of vitamin A **(Yeum & Russell 2002),** and lutein and zeaxanthin have been implicated in preventing macular degeneration **(Landrum & Bone 2004).** The concentrations of total flavonoids. Phenolic compounds , carotenoids indicate that 70 % and 99.9% ethanolic apricot seed extract act as effective antioxidants with high free radical scavenging capacity.

The *in-vivo* anti-inflammatory activity of the extracts was studied using formalin induced paw edema in rats and data were compared with that of control. Oral administration of 70 % and 99.9 % ethanolic extracts in a dose of 100 mg/kg induced a significant decrease in inflamed rat paw thickness. These results matched with previously obtained by **Badr and Tawfik (2010)** who reported that apricot kernel extract possesd marked an anti-inflammatory activity in histamine-induced paw edema in rats. Induction of edema in rat's paw by formalin is a biphasic response, in which the first phase is mediated by histamine, serotonin and kinins and then the second phase is mediated by prostaglandins (cyclooxygenase product of arachidonic acid metabolism) and production of reactive oxygen species (ROS) **(Chen, 1993 & Panthong *et al.*, 2004)**. The effect of apricot seed extract as anti-inflammatory may be attributed by inhibition of pro-inflammatory cytokines and COX-2 synthesis and reduction in prostaglandin synthesis and this was reported by **Chang *et al.*, (2005)** who showed that *Armeniacae semen* exerts anti-inflammatory and analgesic effects by suppression of cyclooxygenase-2 and inducible

nitric oxide synthase expressions. *Armeniacae semen* extract inhibited LPS-stimulated enhancement of COX-2 enzyme activity and PGE2 production in the mouse BV2 microglial cells. Elevation of COX-2 activity is closely associated with the occurrence of cancers, arthritis, and several types of neurodegenerative disorders. PGE2, a major metabolite of the COX-2 pathway, has emerged as an important lipid mediator of inflammatory and immunoregulatory processes. PGE2 has been implicated in the pathogenesis of acute and chronic inflammatory disease states **(Hinz *et al.*, 2000).** Specific COX-2 inhibitors are also known to attenuate the symptoms of inflammation **(Shao *et al.*, 2000).** From our results we can suggest that apricot seed extract has powerful anti-inflammatory effect.

The analgesic activity of both extracts was determined using both writhing test and hot plate test. In writhing test, the standard group showed 77.37 % protection against writhing induced by glacial acetic acid. Oral administration of 70 % and 99.9 % ethanolic extracts in a dose of 100 mg/kg induced a significant analgesic activity with 68.99 % and 67.88 % protection percentage. In hot plate test, the data showed that both 70 % and 99.9 % ethanolic extracts induced significant analgesic activity. These results matched with previously obtained by **Badr and Tawfik (2010)** who reported that apricot kernel extract had a marked analgesic activity when administered orally. The mechanism of the analgesic action may be due to inhibition of release of endogenous substances that excite pain nerve endings, which is mediated via a peripheral mechanism **(Jing-Rong *et al.*, 2008).**

CONCLUSION

Ethanolic apricot seed extracts are safe and non-toxic with some pharmacological activities as analgesic and anti-inflammatory activities. These effects may be attributed to presence of biochemical ingredients such as phenolic compounds, flavonoids and carotenoids. These date confirm the therapeutic medicinal importance of apricot seeds.

Table 2. The anti-inflammatory effect of 70% and 99.9 % ethanolic extracts of apricot seeds in formalin induced paw edema in rats. (n=5).

Treatment	Dose (mg/kg b.wt.)	Mean of right paw thickness in mm					
		Pre -reatment	1 hours	2 hours	3 hours	4 hours	5 hours
Control	0	0.71 ± 0.02	0.72 ± 0.02	0.79 ± 0.03	0.85 ± 0.02	0.86 ± 0.02	0.86 ± 0.02
Indomethacin (standard)	10	0.71 ± 0.02	0.58 ± 0.01 ^{a}	0.51 ± 0.02 ^{a}	0.5 ± 0.02 ^{ab}	0.48 ± 0.02 ^{ab}	0.5 ± 0.02 ^{ab}
99.9 % ethanolic extract	100	0.71 ± 0.02	0.60 ± 0.02 ^{a}	0.58 ± 0.01 ^{a}	0.56 ± 0.02 ^{b}	0.54 ± 0.01 ^{b}	0.56 ± 0.01 ^{b}
70 % ethanolic extract	100	0.71 ± 0.02	0.56 ± 0.01 ^{a}	0.52 ± 0.02 ^{a}	0.52 ± 0.01 ^{b}	0.53 ± 0.01 ^{b}	0.51 ± 0.01 ^{b}

The values represent the mean ± S.E. of five animals for each group. Values in the column with different superscript letters (a,b) are significantly different at $P<0.05$ when compared with control. Data were analyzed by using One-way ANOVA followed by Duncan test.

Table 3. The peripheral anti-nociceptive activity of the 70% and 99.9 % ethanolic extracts of apricot seeds using acetic acid induced writhing technique in mice.(n=5).

Treatment	Dose (mg/kg b.wt.)	No. of Writhes in 30 min.	% of protection
Control	0	71.6 ± 0.5	0
Diclofenac sodium (standard)	25	16.2 ± 0.6 [b]	77.37 %
99.9 % ethanolic extract	100	23 ± 0.7 [a]	67.88 %
70 % ethanolic extract	100	22.2 ± 0.9 [a]	68.99 %

The values represent the mean ± S.E. of five animals for each group. Values in the column with different superscript letters (a,b) are significantly different at $P<0.05$ when compared with control. Data were analyzed by using One-way ANOVA followed by Duncan test.

Table 4. The central anti-nociceptive activity of the 70% and 99.9 % ethanolic extracts of apricot seeds using hot plate test.

Treatment	Dose (mg/kg b.wt.)	0.5 hours	1 hours	1.5 hours	2 hours
Control	0	7.8 ± 0.8	8.8 ± 0.4	7.4 ± 0.5	7.8 ± 0.8
Ibuprofen (standard)	50	14 ± 0.7 [b]	14 ± 0.6 [b]	16.8 ± 0.6 [b]	16.8 ± 0.5 [b]
99.9 % ethanolic extract	100	12.6 ± 0.5 [a]	13.2 ± 0.6 [a]	13 ± 0.7 [a]	14.8 ± 0.4 [a]
70 % ethanolic extract	100	12.6 ± 0.5 [a]	13.2 ± 0.6 [a]	13.6 ± 0.7 [a]	15 ± 0.7 [a]

The values represent the mean ± S.E. of five animals for each group. Values in each column with different superscript letters (a,b) are significantly different at $P<0.05$ when compared with control. Data were analyzed by using One-way ANOVA followed by Duncan test.

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