

## RESEARCH ARTICLE

# Effect of *Prunus armeniaca* L. Methanol Extracts on Antioxidant Enzyme Activity and Liver Function in Albino Mice

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## ABSTRACT

In this study, we aimed to determine the effect of *Prunus armeniaca* L. methanol extract on antioxidant enzyme activity (AEA) and liver function in albino mice.

**Method:** In the liver, the antioxidant activity was measured by estimating the concentration of glutathione (GSH), catalase (CAT), and Malondialdehyde level (MDA).

**Results:** The liver total catalase level (CAT), glutathione level (GSH), and MDA results were significantly high in mice treated with apricot seeds methanolic extract at three doses (50, 100, and 150 mg/kg) group compared with control groups, while in MDA apricot seeds methanolic extract at a dose (150 mg/kg) non-significantly compared with control groups. Advancement in liver function parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and acitic phosphatase (ACP) compared with control.

**Conclusion:** The results indicate that apricot seeds methanolic extract might remain a potential candidate in preventing diseases.

**Keywords:** Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Acitic phosphatase, Catalase, Glutathione, *Prunus armeniaca* L.

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**Conflict of interest:** None

## INTRODUCTION

The human body has a compound system of natural enzymatic and non-enzymatic antioxidant fortifications, which counteract the harmful effects of allowed radicals and other oxidants. Free radicals are accountable for producing many diseases, including cancer, cardiovascular disease, neural complaints, Alzheimer's disease, mild reasoning impairment, Parkinson's disease, aging, and atherosclerosis.<sup>1</sup> Protection against free radicals can be improved by ample consumption of dietary antioxidants. Moreover, there is a substantial indication indicating that nutrients covering antioxidants and therapeutic plants or their secondary metabolite are of main importance in disease deterrence.

Consequently, antioxidants are of great advantage in improving the excellence of life by preventing or delaying the onset of degenerative diseases due to oxidative stress.<sup>2</sup> The Oxidative stress results from an imbalance between extreme formation of reactive oxygen species (ROS) and/or reactive nitrogen species and partial antioxidant defenses.<sup>3</sup> One of the important antioxidants is flavonoids, which signify a range of polyphenolic compounds naturally happening in plants.<sup>4</sup> Flavonoids are potentially involved in cardiovascular deterrence mainly by decreasing oxidative stress and increasing NO bioavailability; consequently, the estimation of flavonoid gratified in plants plays an important role in protection against ROS.<sup>5</sup>

Phenolic compounds are significant plant chemicals and production essential roles in the living systems. There is substantial interest in polyphenols and carotenoids due to their antioxidant properties and likely ability to alleviate anti-aggregant, anti-ischemic, anti-allergic, anti-mutagenic, and anti-inflammatory in addition to being effective in alleviating cardiovascular diseases. Also, certain functional foods have been associated with improved mental capacity,<sup>6</sup> immunity, and anti-aging benefits. Apricot fruit in this setting may be considered as a functional food taking appreciable quantities of biologically active phytochemicals

Oxidative stresses, because of the production of reactive oxygen species and free radicals reason damage to macromolecules (protein, lipids and nucleic acids) and tissue damages. These conditions lead to pathogenesis and chronic disorders, counting cancer, inflammations, sores, diabetes, and cardiovascular diseases.<sup>7</sup> The antioxidant properties of apricot fruit are credited to its rich phytochemical composition. Numerous studies have exposed the potential of apricot to be measured as a functional food founded on its free radical scavenging actions.<sup>8</sup> The present study aimed to regulate the potential protective role of methanol extract in contradiction of oxidative stress using BALB/c mice *in-vivo*.

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## MATERIALS AND METHODS

### Plant Collection and Extraction

Apricot fruits were collected from Baghdad, from the groves of Al-Taji in May and classified as (*P. armeniaca* L.) at the herbarium of Biology Department at the college. Fruits seeds were air-dried at 37°C and removed their outer shell to extract the kernel. The kernels were powdered using electric blender and 50 grams was extracted with 98% methanol (500 mL) ratio of 1: 5 (w/v) at 65°C for 3 hours by the soxhlet apparatus. The extracts solution was focused on dryness under reduced pressure in a rotating evaporator to yield dried crude extract, frozen at -20°C. The dried extract were dissolved in two drops of dimethyl sulfoxide (DMSO) to the concentration of 10 mg/L.

### Animals and Experimental Design

The study was approved out on Albino male mice (200 ± 25g), ages between (8–10) weeks. The animals were distributed into six groups, all with ten mice (Total:60 mice) and housed in the animal house of Biology Department in College of Education for Pure Science, Ibn Al-haitham, under controlled environmental conditions (12L:12D light cycles; 25°C ± 2 temperature). In this experiment, three doses of Apricot seeds methanolic extract (50, 100 and 150 mg/kg) respectively and injected intraperitoneally (IP) with 0.1 mL/day for 14 days as well as three controls were included: the first was distilled water (normal controls) (0.1 mL/day: Oral dose). The second was Immune suppressive cyclosporine (negative control) (0.1 mL/day: Oral dose), and the third was Interferon alpha (positive control) (0.1 mL/day: subcutaneous dose).

### Determination of Antioxidants

Glutathione and catalase were determined in the liver homogenate rendering to the method via Sedlak and Lindsay (1968),<sup>9</sup> while MDA was resolute in the liver homogenate allowing to the method by Guidet and Shah 1989.<sup>10</sup> The tissue was homogenized in PBS bu\_er at a ratio of 1 g tissue to 4 mL bu\_er, shadowed by centrifugation at 3000 rpm for 10 minutes at 4°C. The supernatant was mixed with DTNB at 1:1 ratio. The absorbance was recorded at 415 nm, and the following equation intended the results:

Glutathione activity (mol mg wet weight<sup>-1</sup>) =  $\left(\frac{AB}{E}\right) \times \frac{L}{mg}$  liver weight  
AB = sample absorbance, E 1360, L = light length.

### Malondialdehyde Level (MDA)

An aliquot (1-mL) of liver tissue homogenate supernatant was transported to 10 mL test tube, though 1-mL of purified water was additional to a second test tube and noticeable as blank. To both tube, 1-mL of TCA (17.5%) was added, trailed by the addition of 1-mL of TBA (0.6%). The tubes were vortex and protected in a water bath at 100°C for 15 minutes, and at that time cooled immediately to room temperature for 10 minutes, followed by an adding of 1 mL of TCA (70%) and shake well and left to stand for 20 minutes at room temperature. Then, the test tubes were centrifuged for 15 minutes at 2000 rpm, and the supernatant was separated and transferred to another test tube. To finish, the absorbance was read at 534 nm. The concentration of MDA was intended by the following equation:

$$\text{MDA Concentration} \left( \frac{\text{nmol}}{\text{mL}} \right) = \left( \frac{\text{Sample Absorbance}}{E_0} \right) \times L \times D$$

E<sub>0</sub> = Extinction coefficient 1.56

L = Light bath 1 cm

D = Dilution volume

### Determination of Catalase

Catalase activity was strong-minded, conferring Sedlak and Lindsay (1968).<sup>9</sup> Briefly, 100\_1 of samples was additional to 1.9 mL of phosphate bu\_er; after that, 1-mL of H<sub>2</sub>O<sub>2</sub> was added to altogether samples and the absorbance was read at 240 nm for 3 minutes.

### Estimation of Enzymes Level in Liver Technique

Automated Device biochemical by FLEXOR.

### Statistical Analysis

Data analysis was approved by the available statistical set of SPSS-27 (Statistical Packages for Social Sciences- version 27). Data were obtainable in simple measures of frequency, percentage, nasty, standard deviation, and range (minimum-maximum values).

The significance of the change of different incomes (quantitative data) was verified using Students' t-test for difference between two independent means or ANOVA test for difference amongst more than two autonomous means. Statistical significance was careful whenever the p ≤ 0.05.

**Table 1:** Treatment effects on the catalase activity in mice liver homogenate.

Mice group	Catalase (mmol/L)		p-value compared with				
	No	Mean ± SD	Control	INF α	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	2.45 ± 0.31	0.0001#	0.151	0.616	0.645	0.638
Apricot 100%	10	2.50 ± 0.01	0.0001#	0.0001#	0.0001#	0.866	
Apricot 50%	10	2.50 ± 0.02	0.0001#	0.0001#	0.0001#		
Cyclosporine	10	2.40 ± 0.06	0.0001#	0.0001#			
INF α	10	2.30 ± 0.04	0.0001#				
Control	10	1.40 ± 0.01					

#Significant difference between two independent means using Students-t-test at 0.05 level.

**RESULTS**

**Determination of Catalase**

The liver total catalase level results (Table 1 and Figure 1) were significantly condensed in mice treated with INF alpha, while they significantly increased in Apricot seeds methanolic extract at three doses (50, 100 and 200 mg/kg) and treated groups in treated cyclosporine compared with control groups.

**Determination of Glutathione**

The liver total glutathione level results (Table 2 and Figure 2) were significantly high in mice treated with cyclosporine, however, they significantly decreased in INF alpha treated and Apricot seeds methanolic extract at three doses (50, 100 and 150 mg/kg) groups. While significant differences were observed between cyclosporine treated, Apricot seeds methanolic extract at three doses (50, 100, and 200 mg/kg) groups and INF alpha treated significant difference compared with control groups.

**Determination of Malondialdehyde (MDA)**

The liver total MDA level results (Table 3 and Figure 3) was significantly high in mice treated with INF alpha, where they significantly decreased in cyclosporine treated and Apricot seeds methanolic extract at three doses (50, 100, and 150 mg/kg) groups. Though significant differences were detected between cyclosporine treated, Apricot seeds methanolic extract at two doses (50 and 100 mg/kg) groups and INF alpha treated significant difference compared with control groups. In contrast, non-significant differences were observed between Apricot seeds methanolic extract doses (150 mg/kg) and control groups.

**Liver Functional Enzymes**

*Glutamate Oxaloacetic Transaminase (GOT)*

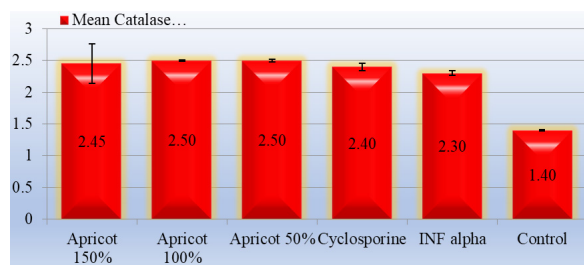
The liver total GOT level results (Table 4 and Figure 4) was significantly high in mice treated with Apricot seeds methanolic extract at two doses (50, and 100 mg/kg) groups. They significantly decreased in INF alpha treated and non-significantly in mice treated with Apricot seeds methanolic extract at doses (150 mg/kg) groups and treated with cyclosporine. Although significant differences were observed, apricot seeds methanolic extract at two doses (50 and 100 mg/kg) groups and INF alpha treated compared with control groups. At the same time, non-significant differences was observed between Apricot seeds methanolic extract at doses (150 mg/kg) group and treated with cyclosporine compared with control groups.

**Glutamate Pyruvate Transaminase (GPT)**

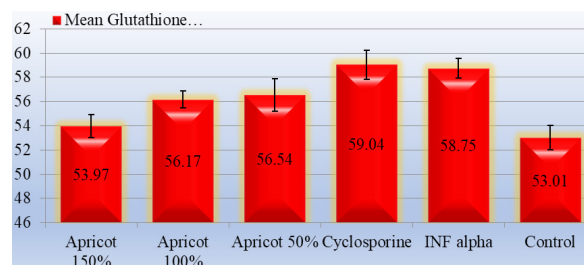
The liver total GPT level results (Table 5 and Figure 5) was significantly high in mice treated with Apricot seeds methanolic extract at two doses (50, and 100 mg/kg) groups. However, they significantly decreased in INF alpha treated and treated with Apricot seeds methanolic extract at doses (150 mg/kg) groups and treated with cyclosporine. Although significant differences were observed, Apricot seeds methanolic extract at three doses (50,100 and 150 mg/kg) groups, INF alpha treated and cyclosporine treated, compared with control groups.

**Alkaline Phosphatase (ALP)**

The liver total ALP level results (Table 6 and Figure 6) was significantly high in mice treated with Apricot seeds methanolic extract at two doses (50, 100 and 150 mg/kg) groups, although they significantly decreased in INF alpha treated and treated with cyclosporine Although significant differences



**Figure 1:** Treatment effects on the catalase activity in mice liver homogenate



**Figure 2:** Treatment effects on the glutathione activity in mice liver homogenate

**Table 2:** Treatment effects on the glutathione activity in mice liver homogenate.

Mice group	Glutathione (mmol/L)		p-value compared with				
	No	Mean ± SD	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	53.97 ± 0.93	0.041#	0.0001#	0.0001#	0.0001#	0.0001#
Apricot 100%	10	56.17 ± 0.71	0.0001#	0.0001#	0.0001#	0.459	
Apricot 50%	10	56.54 ± 1.36	0.0001#	0.0001#	0.0001#		
Cyclosporine	10	59.04 ± 1.20	0.0001#	0.531			
INF alpha	10	58.75 ± 0.81	0.0001#				
Control	10	53.01 ± 1.01					

#Significant difference between two independent means using Students-t-test at 0.05 level.

were observed, apricot seeds methanolic extract at three doses (50, 100, and 150 mg/kg) groups, treated with cyclosporine and INF alpha treated compared with control groups.

**Acitic Phosphatase (APC)**

The liver total APC level results (Table 7 and Figure 7) was significantly high in mice treated with Apricot seeds methanolic extract at two doses (100 and 150 mg/kg) groups and INF α. They significantly decreased in treated with cyclosporine and Apricot seeds methanolic extract at doses (50 mg/kg). Although significant differences were observed, apricot seeds methanolic extract at three doses (50, 100 and 150 mg/kg) groups, treated with cyclosporine and INF α treated compared with control groups.

Lipid peroxidation is one value of oxidative stress helped by high intracellular concentrations of ROS. Thr MDA is a well-known biomarker of oxidative stress formed by the reaction of polyunsaturated fatty acid peroxidation.

Determination of MDA level is apposite and reliable method for presentation tissue damage degree. The MDA level was make significantly increased via intravenously managed Oleic acid. Liu *et al.* stated that after oleic acid injection, the level of ROS in lung tissues expeditiously increased and reached peak level in 30 minutes.<sup>11</sup> Likewise, Hshing *et al.*

reported that serum MDA level was significantly increased by oleic acid infusion within 30 minutes.<sup>12</sup> Yang *et al.* showed that infusion of increasing doses of oleic acid markedly increased the MDA level of bronchoalveolar lavage fluid (BALF) in guinea pigs.<sup>13</sup>

Vardi *et al.*<sup>14</sup> conveyed that apricot was found to be additional effective than β-carotene increase in SOD, CAT and GSH levels and reduction in MDA levels of the duodenal tissue. Though both studies (current and theirs) are extremely unlike from another, these findings in MDA, CAT, and GSH levels of liver and intestine have scientific importance due to the oxidant/antioxidant balance in service of oxidants.

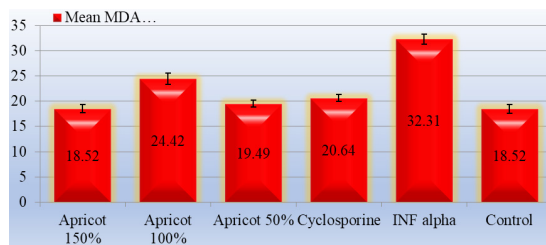
The liver is the main site of detoxification and the primary mark of drug exposure in the body. High levels of drugs source various hepatic disorders via producing pro-oxidants/reactive oxygen species (ROS), which can induce cellular injury in various ways via affecting the cellular biomolecules, such as lipids, DNA, and proteins.<sup>15</sup> In this study, the results showed increase in functional liver enzymes (GPT, GOT, ALP and ACP).

The hepatoprotective result of dried apricot seeds was appraised by measuring the level of serum liver harm marker enzymes (AST, ALT, GGT and LDH) which increased because of hepatic damage induced by alcohol and decreased in groups of

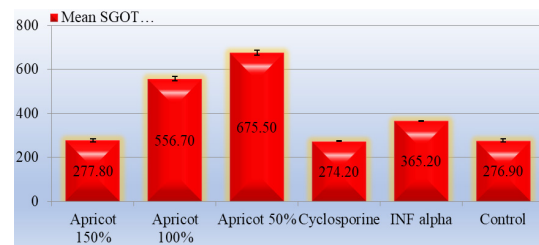
**Table 3:** Treatment effects on MDA activity in mice liver homogenate.

Mice group	No	MDA (nmol/L)		p-value compared with			
		Mean ± SD	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	18.52 ± 0.77	0.998	0.0001#	0.0001#	0.009#	0.0001#
Apricot 100%	10	24.42 ± 1.15	0.0001#	0.0001#	0.0001#	0.0001#	
Apricot 50%	10	19.49 ± 0.69	0.014#	0.0001#	0.001#		
Cyclosporine	10	20.64 ± 0.66	0.0001#	0.0001#			
INF α	10	32.31 ± 0.97	0.0001#				
Control	10	18.52 ± 0.88					

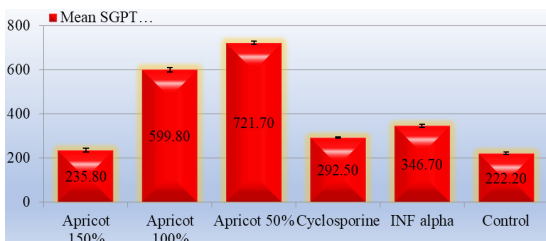
#Significant difference between two independent means using Students-t-test at 0.05 level.



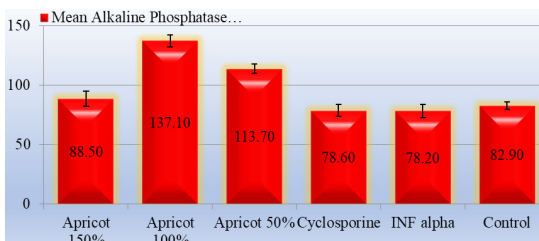
**Figure 3:** Treatment effects on MDA activity in mice liver homogenate.



**Figure 4:** Treatment effects on GOT activity in mice liver homogenate



**Figure 5:** Treatment effects on GOT activity in mice liver homogenate



**Figure 6:** Treatment effects on ALP activity in mice liver homogenate

animals complemented with diet containing dried apricot seeds.<sup>16</sup> Nourishing of ground apricot kernel (1.5 mg/kg) for 4 weeks has better liver fibrosis caused by dimethylnitrosamine which might be used as a therapeutic or defensive measure against hepatic fibrosis.<sup>17</sup> The apricot kernel administration consumes improved biochemical values because of increase levels of oleic acid, and other polyphenols in apricot seeds.

During the study Ramadan *et al.* (2020) Apricot kernels possess hepatoprotective and anticancer actions that justify its traditional use, and its possible for the treatment of liver diseases counting hepatocellular carcinoma, Pretreatment of the rats with 70 and 99.9% ethanolic apricot kernels extracts (100 mg/kg), amygdalin and silymarin (50 mg/kg) banned elevation in liver function parameters e.g., ALT, AST, ALP caused via carbon tetrachloride inoculation with a significant increase in albumin, total proteins, and no result on total direct bilirubin when

compared to persons in hepatotoxic group, both extracts also showed anticancer activity against hepatocellular carcinoma via diminishing the raised serum levels of AST, ALT, ALP.<sup>18</sup>

In study, Huo *et al.* (2011) assessed the antihepatotoxic effect of aqueous licorice extract (LE) on the carbon tetrachloride (CCl4)-induced liver damage in a rat model. Hepatic damage, the increased actions of serum AST, ALT, ALP actions, and decreased levels of serum total protein (TP), albumin (Alb) and globulin (G) was induced in rats by management of CCl4 at 3 mL/kg. Licorice extract significantly reserved the raised AST, ALP and ALT activities and the reduced TP, Alb and G levels produced via CCl4 intoxication. It also improved liver superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), Glutathione S-transferase (GST) activities and glutathione (GSH) level, reduced malondialdehyde (MDA) level.<sup>19</sup>

**Table 4:** Treatment effects on GOT activity in mice liver homogenate

Mice group	No	SGOT (U/L)		p-value compared with			
		Mean ± SD (Range)	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	277.8 ± 6.94	0.774	0.0001#	0.132	0.0001#	0.0001#
Apricot 100%	10	556.7 ± 10.10	0.0001#	0.0001#	0.0001#	0.0001#	
Apricot 50%	10	675.5 ± 10.58	0.0001#	0.0001#	0.0001#		
Cyclosporine	10	274.2 ± 1.93	0.246	0.0001#			
INF alpha	10	365.2 ± 2.15	0.0001#				
Control	10	276.90 ± 6.85					

#Significant difference between two independent means using Students-t-test at 0.05 level.

**Table 5:** Treatment effects on GOT activity in mice liver homogenate

Mice group	No	SGPT (U/L)		p-value compared with			
		Mean ± SD	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	235.80 ± 7.64	0.0001#	0.0001#	0.0001#	0.0001#	0.0001#
Apricot 100%	10	599.80 ± 10.03	0.0001#	0.0001#	0.0001#	0.0001#	
Apricot 50%	10	721.70 ± 7.87	0.0001#	0.0001#	0.0001#		
Cyclosporine	10	292.50 ± 3.17	0.0001#	0.0001#			
INF alpha	10	346.70 ± 7.48	0.0001#				
Control	10	222.20 ± 6.16					

#Significant difference between two independent means using Students-t-test at 0.05 level.

**Table 6:** Treatment effects on ALP activity in mice liver homogenate

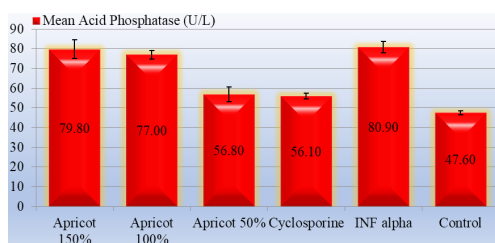
Mice group	No	Alkaline phosphatase (U/L)		p-value compared with			
		Mean ± SD	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	88.50 ± 6.54	0.026 #	0.001 #	0.001 #	0.0001#	0.0001#
Apricot 100%	10	137.10 ± 5.30	0.0001#	0.0001#	0.0001#	0.0001#	
Apricot 50%	10	113.70 ± 3.95	0.0001#	0.0001#	0.0001#		
Cyclosporine	10	78.60 ± 4.90	0.032 #	0.867			
INF alpha	10	78.20 ± 5.59	0.033 #				
Control	10	82.90 ± 3.21					

#Significant difference between two independent means using Students-t-test at 0.05 level.

**Table 7:** Treatment effects on APC activity in mice liver homogenate

Mice group	Acid Phosphatase (U/L)			p-value compared with			
	No	Mean ± SD	Control	INF alpha	Cyclosporine	Apricot 50%	Apricot 100%
Apricot 150%	10	79.80 ± 4.76	0.0001#	0.538	0.0001#	0.0001#	0.109
Apricot 100%	10	77.00 ± 2.21	0.0001#	0.003 #	0.0001#	0.0001#	
Apricot 50%	10	56.80 ± 3.79	0.0001#	0.0001#	0.595		
Cyclosporine	10	56.10 ± 1.52	0.0001#	0.0001#			
INF alpha	10	80.90 ± 2.85	0.0001#				
Control	10	47.60 ± 0.97					

#Significant difference between two independent means using Students-t-test at 0.05 level.

**Figure 7:** Treatment effects on APC activity in mice liver homogenate

In study, Al-Shmgani *et al.* (2019) made it optional that the aqueous extract of *Amblyomma maculatum* leaves have strong antioxidant activity and cytotoxicity in contradiction of cell line with potential pro-inflammatory activity.<sup>20</sup>

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