ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Utilization of Quince *(Cydonia oblonga)* Peel and Exploration of Its Metabolite Profiling and Cardioprotective Potential Against Doxorubicin-Induced Cardiotoxicity in Wistar Rats

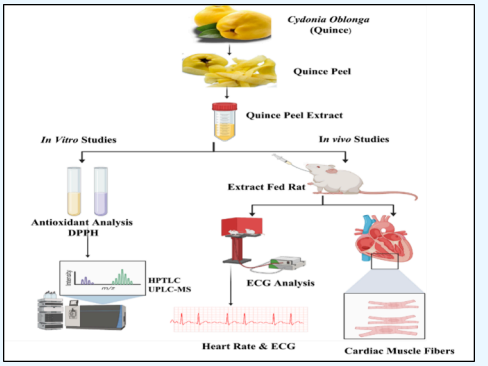
Entesar Hanan, Nazeer Hasan, Sultan Zahiruddin, Sayeed Ahmad, Vasudha Sharma,* and Farhan J Ahmad*

Cite This: *ACS Omega* 2023, 8, 40036−40050

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Quince (*Cydonia oblonga* Mill.) is a pomaceous fruit that is typically processed into jams, jellies, and marmalade. The byproduct, i.e., the quince peel emanated from the processing industry, can be upcycled, ensuring zero waste policy and resulting in a sustainable food system. In our study, the quince peel was explored for *in vitro* phytochemical analysis and *in vivo* cardioprotective potential. Two diverse extractions (ultrasonication and reflux) and four different solvents (aqueous, ethanolic, hydroethanolic, and methanolic) were used for the extraction of quince peel and assessed for the phytochemical and antioxidant study. Among all the evaluated extracts, hydroethanolic quince extract extracted through the reflux extraction method showed the maximum phenolic (27.23 ± 0.85 mg GAE/g DW) and flavonoid (16.5 ± 1.02 mg RE/g DW) content. The maximum antioxidant potential (DPPH) with an IC_{50} value of 204.8 ± 2.24 μg/mL was noted for the hydroethanolic extract. This best active extract was then subjected to HPTLC, UPLC−MS, mineral, and FTIR analysis to study the metabolic profiling and inorganic composition and to confirm the presence of bioactives. Additionally, the *in vivo* study was done in rats using doxorubicin (DOX)-induced cardiotoxicity. The rats were given extracts orally at 160 and 320 mg/kg bw for 30 days. ECG analysis was done at the termination of the experiment. Besides this, the lipid profile, blood serum parameters (CK-MB, LDH, AST), and tissue parameters (MDA, SOD, GSH, CAT) were analyzed. The DOX-treated group unveiled a substantial variance ($p < 0.001$) in all the parameters in contrast to the normal control group and extract control groups. However, the pretreated groups substantially alleviated the DOX-induced changes in all the parameters. Additionally, recuperation in histopathological alterations of the cardiac tissue in contrast to the DOX-induced toxicity was also seen in the pretreated groups. Thus, it could be said that the cardioprotective activity of the quince peel extract attributed to the presence of phytoconstituents counteracted the DOX-induced cardiotoxicity and assisted in the restoration of the cardiac injury in rats.



1. INTRODUCTION

Oxidative stress that results from the free radical and reactive oxidative species (ROS) formation is responsible for various chronic disease, viz., atherosclerosis, cardiovascular disease, cancer, and other neurodegenerative disorders.[1] As per the World Health Organization (WHO), cardiovascular disease (CVD) is foreseen to be the foremost reason for mortality by 2030.[2] Doxorubicin (DOX) is an anticancer anthracycline drug reported to produce cardiotoxicity.[3] The cardiotoxicity caused by DOX has been explained by numerous molecular mechanisms, viz., oxidative stress, metabolism of iron, dysregulation of Ca2+ homeostasis, modulation of gene expression, modifications of sarcomere structure, and cell death.[4] The side effects of DOX resulting in cardiotoxicity adapted from Rawat et al. are shown briefly in Figure 1.[5]

The positive effect of numerous medicinal plants on DOX-induced cardiotoxicity is well documented in the literature.[6] The compounds obtained from plants such as phenolics are reported to be of great pharmacological importance having antioxidant, antihypertensive, antiatherosclerosis, antidiabetic, and anticancer properties. They are also said to alleviate the risk of CVD.[7] The consumption of bioactive-rich compounds helps reduce the oxidative stress and hence aids in the reduction of chronic diseases. Quince (*Cydonia oblonga*), belonging to the family Rosaceae, is an underutilized fruit that is promulgated to be a rich source of bioactive compounds.[8,9] It is indigenous to Iran and Turkey. In India, quince fruit is found in a few parts of Himachal Pradesh and Jammu and

Received: February 2, 2023
Accepted: August 24, 2023
Published: October 19, 2023

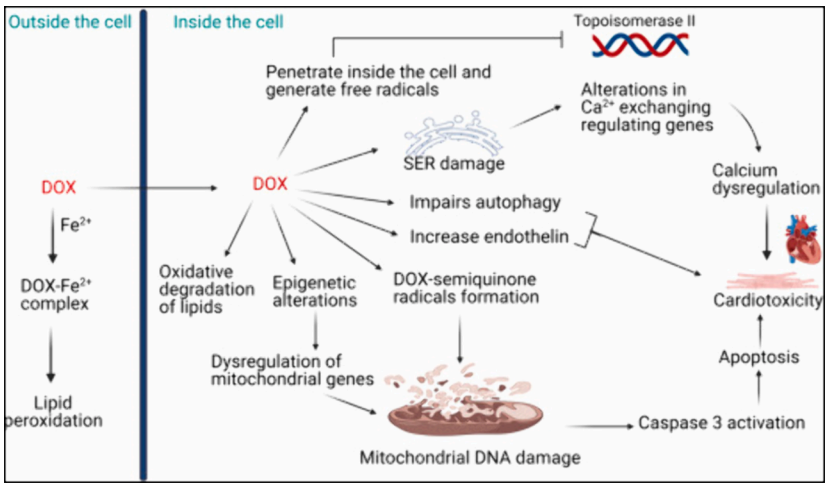


Figure 1. Different mechanisms of DOX-induced cardiotoxicity (source: Rawat et al., 2021[5]).

Kashmir (J&K), where it is popularly known as "Bamchount".[10] Quince is a highly aromatic, astringent, and hard flesh fruit, which makes it unacceptable for fresh consumption and hence makes it an important raw material for the food industry, which processes it into jams, jellies, and marmalades. The waste emanating from the industry, i.e., the peel, is left unused as agricultural waste. This waste can be valorized for the exploration of the bioactive-rich compounds. Nowadays, because of the increasing population, the exploration of new possible sources of bioactive components in the context of health promotion is an important research field. Waste utilization can thereby assist in the enhancement of human welfare and also assist in the alleviation of environmental problems, which is a vital footstep in the direction of accomplishment of Sustainable Development Goals (SDG). The peels of different fruits are said to be a rich source of antioxidants that can be exploited industrially. The peels of fruits are reported to have higher minerals and phytochemicals and can be explored as a pharmaceutical or a food additive.[11] The peels of mango, pomegranate, banana, and watermelon have been reported to be very rich in bioactive components.[12] Quince peels are also reported to be a rich source of polyphenols and antioxidants. Quince peel is reported to possess a higher level of antioxidants compared to the pulp and seed.[13] Quince peel is reported to contain caffeoylquinic acids, quercetin, and derivatives of kaempferol glycosides. Quercetin 3-*O*-rutinoside (rutin) is reported to be the chief polyphenol in the peel (36%).[14] The organic acids reported to be present in the quince peel comprise oxalic, citric, ascorbic, malic, quinic, shikimic, and fumaric acid.[15]

Quince fruit is found to have a good phenolic and antioxidant profile. The reported pharmacological uses of quince fruit include anti-inflammatory, antidiabetic, antiatherosclerosis, antihypertensive, etc.[16] In traditional Uyghur medicine, quince is reported as a cure for CVD, and in Iranian medicine, it is reported as a cure for liver disease, headache, and nausea.[7,17] The cardioprotective effect of quince has been well established by animal studies. Quince leaves are reported to offer a cardioprotective effect against DOX-induced toxicity. Quince leaves are also said to diminish the peroxidation of lipids and oxidative stress in doxorubicin-persuaded cardiac toxicity by means of the presence of the phenolic and flavonoid compounds and the antioxidant activity of leaves.[18,19] Quince is reported to control the level of blood lipids and decrease the blood pressure levels and glucose levels. It is also reported to have a positive effect on thrombosis and body weight.[20] In the current investigation, the cardioprotective potential of quince coproduct, i.e., the peel, was studied against DOX-induced cardiotoxicity in the female Wistar rats. DOX is generally used in breast cancer medication. Henceforth, female Wistar rats were used for the study.[4] Thus, the current study aimed to explore the cardioprotective ability of quince peel in female Wistar rats in contrast to the DOX-induced cardiotoxicity.

2. RESULTS AND DISCUSSION

The extraction process aims to extract the bioactives existing in the plant material. The solvent selection and method of extraction play a critical role in the extraction process.[21] The quince peel was extracted via ultrasonic and reflux extraction using four diverse solvents (water, ethanol, hydroethanol, and methanol). The highest yield in our study was found using organic solvents and a combination of water and an organic solvent. The aqueous extraction gave the lowest yield in both extraction methods, implying that the extraction yield is largely dependent on the solvent polarities. The highest extractive yield in peels in the hydroalcoholic solvent signifies that an increase in the water concentration in the pure solvent enhances the yield.[22,23] The calculated yield is shown in Table 1.

2.1. Estimation of Total Phenols and Flavonoids. The phytochemicals in the plant material, viz., the phenols and the flavonoids, are said to possess antioxidant activity. Their long-term consumption is said to alleviate the risk of several

Table 1. Percent Yield of Peel Extracts by Ultrasonic and Reflux Extraction in Different Solvents[a]

s. no.	extract	ultrasonic extraction (%)	reflux extraction (%)
1	aqueous	21.46 ± 1.71	27.43 ± 1.66**
2	ethanolic	36.36 ± 1.85	31.37 ± 1.56*
3	hydroethanolic	37.43 ± 1.60	35.26 ± 1.75^{ns}
4	methanolic	38.56 ± 1.70	30.40 ± 1.65***

[a]Data are expressed as mean ± SD (n = 6). One-way ANOVA followed by Tukey's multiple comparisons test was used for statistical analysis. Compared to ultrasound extraction: ***p < 0.001, **p < 0.01, *p < 0.05, and ^{ns}p > 0.05.

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

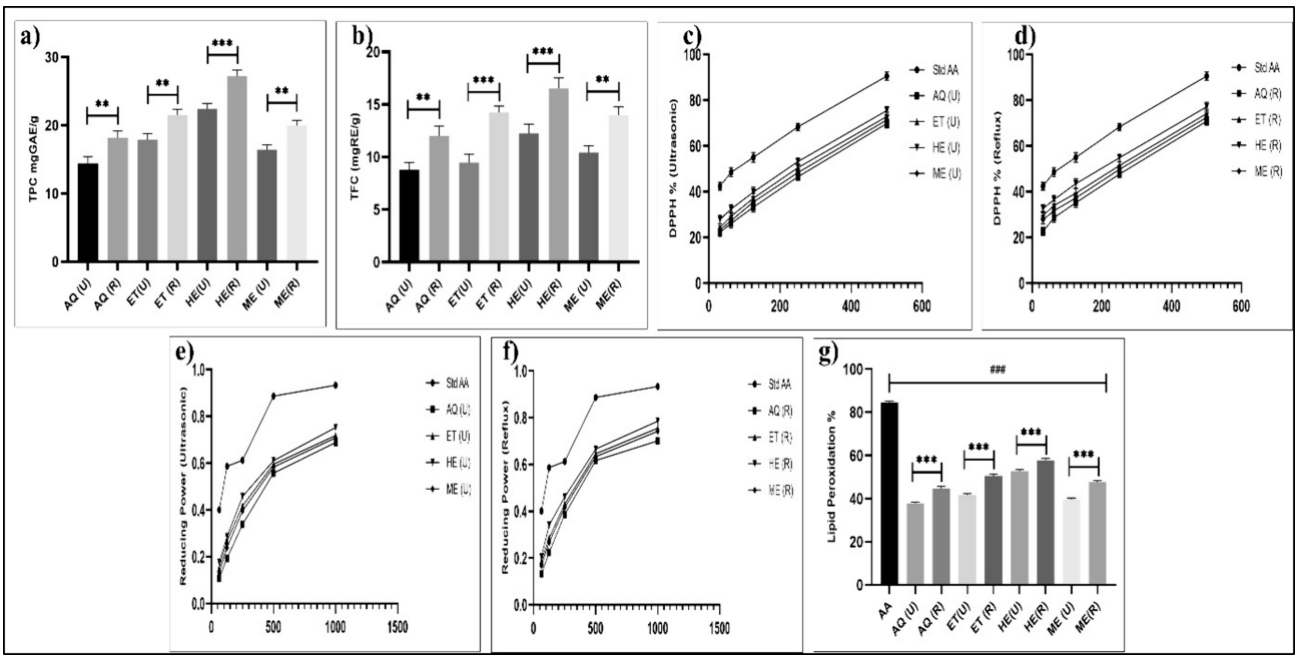


Figure 2. (a) TPC and (b) TFC. Data are shown as (n = 3) mean ± SD, ANOVA (one-way), along with Tukey's multiple comparison test. In comparison to ultrasonic extraction: The significance level was statistically expressed at ***p < 0.001 and **p < 0.01. (c) DPPH % scavenging activity ultrasonic extraction and (d) DPPH % scavenging activity reflux extraction. Data are shown as (n = 3) mean ± SD. (e) Reducing power ultrasonic extraction and (f) reducing power reflux extraction. Data are shown as (n = 3) mean ± SD. (g) Lipid peroxidation %. Data are shown as (n = 3) mean ± SD, ANOVA (one-way), along with Tukey's multiple comparisons test. In comparison to standard ascorbic acid: The significance level was statistically expressed at $^{###}p$ < 0.001. In comparison to the ultrasonic extraction: The significance level was statistical expressed at ***p < 0.001.

inflammatory diseases. The phenols and flavonoids are the naturally occurring phytochemicals that account for the antioxidant activity of the plant. The TPC of the extracts in our study was found to be the least in aqueous extract, 14.41 ± 1.00 mg GAE/g DW, and the highest in hydroethanolic extract, 27.23 ± 0.85 mg GAE/g DW. In both the extraction methods, similar results were found, with the highest and lowest TPC found in the hydroethanolic and aqueous extract, respectively. Further, the trend in other extracts was found to be ethanolic > methanolic > aqueous. The flavonoids also showed a similar trend as phenols. The maximum and minimum flavonoid content was obtained in hydroethanolic extract, 16.5 ± 1.02 mg RE/g DW extracted via reflux extraction, and aqueous extract, 8.7 ± 0.71 mg RE/g DW extracted via ultrasonic extraction, respectively. Thus, the TFC in the hydroethanolic extract was the highest and that in aqueous extract was the lowest in both the methods of extraction. However, the trend in all the other extracts was found to be ethanolic > methanolic > aqueous. The highest phenol and flavonoid contents in quince peel were found in the hydroethanolic extract. Moreover, the values in the heat reflux extraction were found to be higher than those in the ultrasonic extraction, which imply that temperature has a notable influence on the polyphenol extraction owing to the weakening of phenolic interactions at higher temperatures that results in the increase in the diffusion rate of phenolics. In et al. also confirmed the maximum abstraction of phenols and flavonoids from quince peels in the hydroethanolic extract, describing it as the most suitable solvent for phenolic and flavonoid extraction.[24] Similarly, Alothman et al. confirmed that hydroalcoholic extraction can be commonly used for the phenolic extraction from pineapple, banana, and guava due to its ability to dissolve a wide range of phenols.[25] Thus, it could be said that hydroalcoholic solvent is a better choice for the phenolic and flavonoid extraction from peels. A significant difference (p < 0.01−p < 0.001) was obtained among the solvent extracts of ultrasound and reflux extraction as shown in Figure 2a,b.

2.2. Estimation of Antioxidant Activity. Quince peel is an abundant source of secondary metabolic compounds such as phenols and flavonoids, which contribute to the antioxidant activity. 2,2-Diphenyl-1-picryl-hydrazyl radical (DPPH), with reduced power activity in comparison to the standard ascorbic acid, was used to evaluate the antioxidant activity of the quince peel. The DPPH scavenging potential displayed a dose-dependent pattern as it showed an increase with the increase in the concentration of extract as shown in Figure 2c,d. The DPPH activity was found to be the highest in the hydroethanolic extract with a scavenging potential of 75.5 ± 1.83 and 77.2 ± 1.93% in the ultrasonic and heat reflux extraction, respectively. The trend in other extracts was found to be ethanolic > methanolic > aqueous. Thus, in our study, the highest antioxidant activity at 500 μg/mL of the quince peel was 77.2 ± 1.93%, and that of standard ascorbic acid was 90.56 ± 1.82%. The IC_{50} was found to be 204.8 ± 1.98 and 83.79 ± 2.08 μg/mL, respectively.

In the reducing power assay, the greater the absorbance is, the greater is the reductive ability, which in turn is indicative of strong antioxidant potential. The reductive ability of extracts unveiled a dose reliant activity as displayed in Figure 2e,f. The maximum reducing power was recorded in the hydroethanolic extract with a scavenging potential of 0.753 ± 0.078 and 0.785 ± 0.056 in the ultrasonic and heat reflux extraction, respectively. The trend in other extracts was found to be

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

ethanolic > methanolic > aqueous. Thus, the reductive capability at 1000 μg/mL of the quince peel was about 0.785 ± 0.056, and that of the standard ascorbic acid was 0.934 ± 0.041. The reductive potential at 1000 μg/mL in the extract was observed to be comparable to the standard ascorbic acid at 500 μg/mL.

Overall, the peel extracts obtained using organic and hydroethanolic solvent showed a better antioxidant capacity compared to the pure aqueous extract in both the antioxidant assays. The antioxidant activity is mostly reliant on the phenols that act as quenchers and chelators and hence result in an increased scavenging potential.[26] This is in line with our study, as the highest phenolic content was observed in thehydroethanolic extract. A similar finding for the antioxidant activity was reported by other researchers.[25,27]

2.3. Lipid Peroxidation Assay. The use of antioxidants is said to reduce the oxidative stress caused by the peroxidation of lipids. Lipid peroxidation allegedly causes cellular impairment mediated through a radical chain reaction that is one of the leading sources of several diseases. The lipid source, i.e., the egg yolk incubated in $FeSO_4$ presence, causes the development of lipid peroxides that are responsible for numerous pathological events including inflammation and cell impairment.[28] The use of quince peel extract was studied to lessen the oxidative stress triggered by lipid peroxidation. The lipid peroxidation inhibition of the peel extract in contrast to the standard ascorbic acid was analyzed at a concentration of 1000 μg/mL (Figure 2g). The entire group showed a substantial statistical variance ($p < 0.001$) in contrast to ascorbic acid standard. The highest lipid peroxidation inhibition of 52.69 ± 0.764 and 57.73 ± 0.88% was found in the hydroethanolic extract by means of ultrasonic and heat reflux extraction, respectively. The trend obtained in the rest of the extracts was ethanolic > methanolic > aqueous as evident in Figure 2g. The lipid peroxidation inhibition in the hydroethanolic peel extract was found to be the highest, which could be attributed to its higher phenol and antioxidant activity in contrast to the other extracts. The aqueous extract exhibited the lowest lipid per oxidation inhibition concomitant with the result reported by other researchers.[29,30]

2.4. Mineral Analysis of the Best Active Extract. Quince peel extract is a virtuous mineral source. The macrominerals found in the majority include potassium (3.012 ± 0.261 mg/g) followed by magnesium and calcium (0.640 ± 0.076 and 0.200 ± 0.058 mg/g, respectively). In comparison to the other macronutrients, sodium and chlorine were reported in comparatively minor amounts (0.089 ± 0.015 and 0.093 ± 0.013 mg/g, respectively). In micronutrients, the maximum manifestation was found for iron (92.01 ± 0.95 μg/g), manganese (48.04 ± 0.621 μg/g), and zinc (17.02 ± 0.710 μg/g). Others, viz., copper and boron, were also found in significantly lesser amounts (6.04 ± 0.975 and 5.01 ± 0.58 μg/g, respectively). However, minerals like nickel, molybdenum, and cobalt were found in comparatively smaller amounts as given in Table 2. Thus, the mineral analysis of the BAE revealed potassium (K) as the copious mineral found in the extract, which is allegedly responsible for the normal cellular functioning. Among the microminerals, iron (Fe) was found in the highest amount, which is an important constituent of several proteins, such as hemoglobin. In et al. also reported the higher predominance of macronutrient potassium and micronutrient iron in the quince peel.[24]

Table 2. Macro- and Micronutrients in BAE

s. no.	macrominerals	concentration (mg/g)
1	calcium	0.200 ± 0.058
2	magnesium	0.640 ± 0.076
3	sodium	0.089 ± 0.015
4	potassium	3.012 ± 0.261
5	chlorine	0.093 ± 0.010
	microminerals	**concentration** (μg/g)
6	iron	92.01 ± 0.951
7	manganese	48.04 ± 0.621
8	copper	5.01 ± 0.580
9	zinc	17.02 ± 0.710
10	molybdenum	0.09 ± 0.065
11	boron	6.04 ± 0.975
12	nickel	0.051 ± 0.012
13	cobalt	0.06 ± 0.010

2.5. FTIR of the Best Active Extract. FTIR spectroscopy is a reliable procedure for the identification and quantification of the functional compound. The FTIR spectra revealed the incidence of the chemical fingerprint of the bioactives present in the BAE. Figure 3a shows the FTIR spectrum of BAE. The appearance of diverse metabolites is specified by the quantified peaks. The extract showed some prominent broad and sharp peaks at 3280.33, 2934.60, 1722.63, and 1632.93 cm^{-1}. The fingerprint region ranging from 1400 to 900 cm^{-1} revealed the many characteristic single bands of low intensity that are accredited to definite functional groups. The extract showed a prominent major absorption band at 3280.33 cm^{-1} in the region of 3375−3260 cm^{-1}, representative of the occurrence of a polymeric hydroxyl group (O−H), distinctive of polyphenol compounds. In the 2940−2925 cm^{-1} range, the peak at 2934.60 cm^{-1} signifies irregular and proportioned −CH and $-CH_2$ stretch vibrations, ensuing the presence of carbohydrates and sugars in the extracts. At 1722.63 cm^{-1}, the carbonyl group peak was observed that verified the flavanone or terpenoid occurrence.[31] Additionally, at ∼1200 cm^{-1}, the phenolic C−O stretching was observed, which is representative of flavonoid C-rings. The fingerprint region ranging from 1400 to 900 cm^{-1} comprises the many characteristic single bands of low intensity that are accredited to precise functional groups like C−H, C−O, C−N, and P−O bonds. In the fingerprint area, the peaks (1580−1615 and 1450−1510 cm^{-1}) are ascribed to the C═C−C aromatic ring stretching and (670−900 and 950−1225 cm^{-1}) to numerous out-of-plane aromatic C−H bending and in-plane bending, respectively.[32]

2.6. HPTLC of the Best Active Extract. HPTLC fingerprinting is commonly used to separate metabolites from any plant extract. For the quantitative estimation of polyphenols present in the BAE, a number of mobile phase mixtures were explored to separate metabolites present in the plant extract. By means of mobile phase in a ratio of 11:1.1:1.1 (ethyl acetate/formic acid/acetic acid), the separation of metabolites was maximized, and good resolution bands and peaks were obtained. A TLC scanner III chamber was used to analyze the TLC plate under UV at wavelengths of 254 and 366 nm. Ten metabolites were detected after scanning at 254 and 366 nm. In the peel extract, the average quantity of rutin was observed to be 4.9 ± 0.07 μg/mg. Figure 3 displays the TLC chromatograms.

2.7. Metabolite Identification Using UPLC−MS in the Best Active Extract. For the identification of metabolites in

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

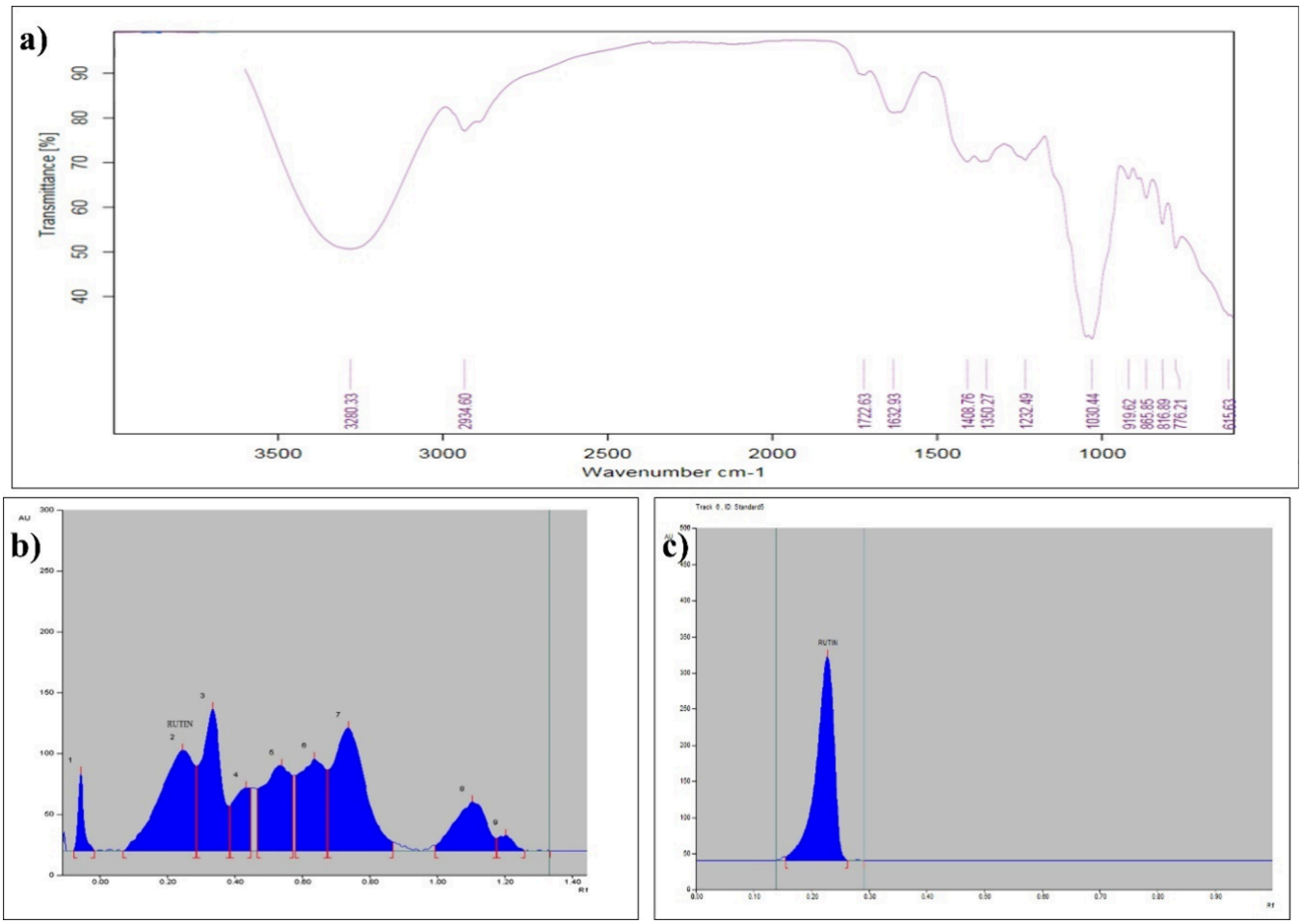


Figure 3. (a) FTIR of BAE. (b) Chromatogram of BAE sample at 264 nm. (c) Chromatogram of standard at 264 nm.

Table 3. Identified Metabolites in the BAE Using UPLC−MS

s. no.	Rt	*m/z* observed	*m/z* theoretical	compound name	compound formula	mass ID
1	0.755	129.233	129.078	dl-pipecolic acid	$C_6H_{11}NO_2$	849
2	0.755	289.388	290.27100	catechin	$C_{15}H_{14}O_6$	73160
3	0.755	289.388	290.27100	epicatechin	$C_{15}H_{14}O_6$	72276
4	0.755	325.299	326.19943	ajmaline	$C_{20}H_{26}N_2O_2$	6100671
5	0.798	133.140	134.02151	malic acid	$C_4H_6O_5$	525
8	1.966	183.038	182.057	dihydrocaffeic acid	$C_9H_{10}O_4$	308986
9	1.996	335.191	336.12357	berberine	$C_{20}H_{18}NO_4^+$	2353
11	1.996	152.356	152.04730	vanillin	$C_8H_8O_3$	1183
12	3.072	431.586	432.38101	kaempferol-3-*O*-rhamnoside	$C_{21}H_{20}O_{10}$	5835713
13	4.04	201.372	202.12051	sebacic acid	$C_{10}H_{18}O_4$	5192
14	4.04	175.289	176.04730	7-hydroxy-4-methylcoumarin	C10H8O3	5280567
15	4.07	228.274	228.07864	resveratrol	$C_{14}H_{12}O_3$	392875
16	4.833	611.397	610.52100	rutin	$C_{27}H_{30}O_{16}$	5280805
17	6.925	116.759	116.01100	fumaric acid	$C_4H_4O_4$	444972
18	6.925	116.759	116.08373	1-hexanoic acid	$C_6H_{12}O_2$	8892
19	6.925	318.243	318.23700	myricetin	$C_{15}H_{10}O_8$	5281672
20	8.235	214.099	214.11061	harmaline	$C_{13}H_{14}N_2O$	3564
21	9.762	611.523	610.56500	hesperidin	$C_{28}H_{34}O_{15}$	10621
22	10.908	301.421	302.04266	quercetin	$C_{15}H_{10}O_7$	5280343
23	10.908	149.142	150.01643	l-(+)-tartaric acid	$C_4H_6O_6$	444305
24	11.776	122.240	122.036	benzoic acid	$C_7H_6O_2$	243
25	12.470	282.520	282.08920	2′,5-dimethoxyflavone	$C_{17}H_{14}O_4$	4231835
26	12.895	117.200	118.02661	succinic acid	$C_4H_6O_4$	1110
27	12.895	117.200	118.06299	alpha-hydroxyisovaleric acid	$C_5H_{10}O_3$	99823

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

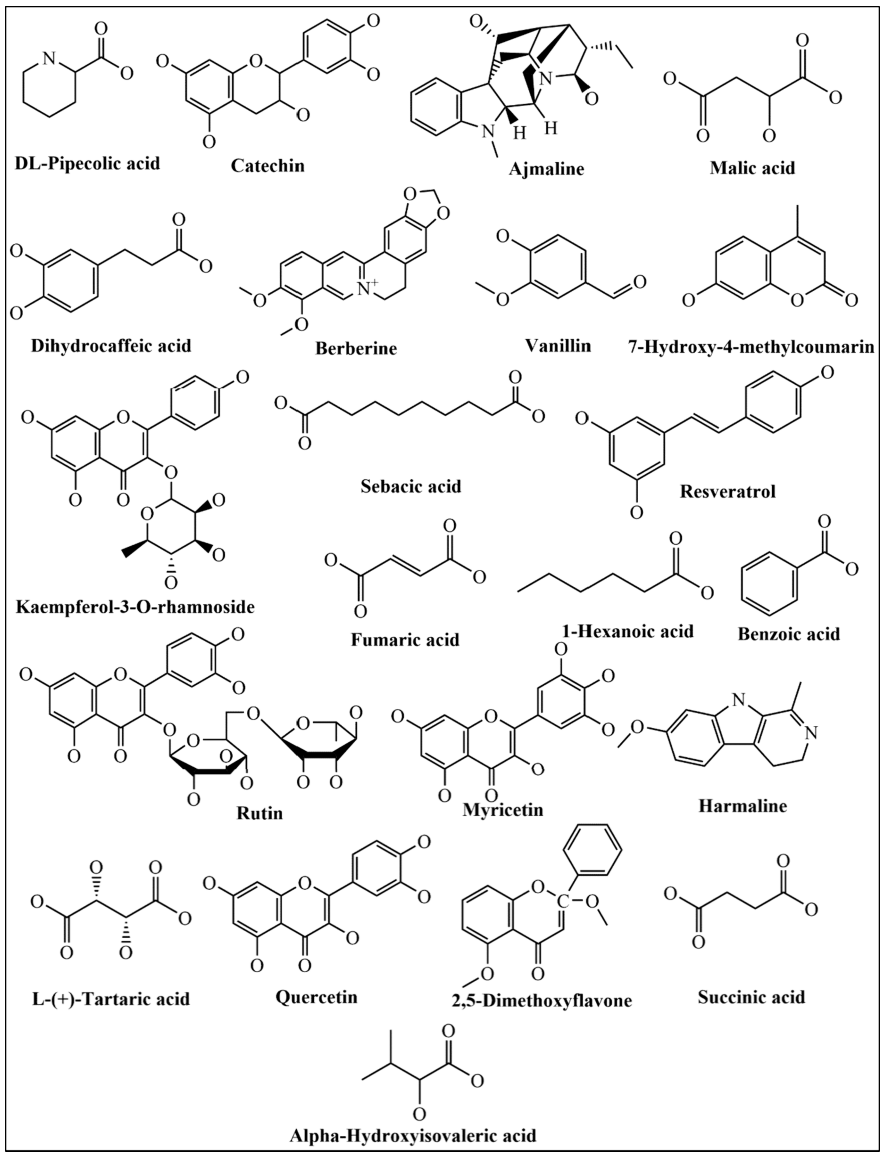


Figure 4. Structure of metabolites identified in BAE using UPLC−MS.

the BAE, UPLC−MS was done. The main metabolites found in the extract are shown in Table 3, and structures are shown in Figure 4. On examination of the UPLC−MS chromatogram, diverse peaks were identified representative of the occurrence of 27 phytochemical components fitting in the alkaloid, flavonoid, and phenol groups. The compounds identified include quercetin, rutin, kaempferol-3-*O*-rhamnoside, catechin, epicatechin, malic acid, fumaric acids, and succinic acid. Several researchers described the occurrence of quercetin, rutin, and kaempferol in the quince peel responsible for the antioxidant and anti-inflammatory properties.[15,33] The existence of ascorbic, citric, fumaric, l-shikimic, malic, and quinic acid in the quince peel was also confirmed.[15] Karar et al. in their study revealed the incidence of 34 polyphenols in quince fruit via LC−MS analysis.[34] The major ones identified include rutin, kaempferol 3-*O*-rhamnoside, catechin, epicatechin, and their derivatives, which are similar to our reported findings. Apart from this, the presence of coumaroylquinic acid derivatives, kaempferol 3-*O*-rutinoside, quercetin-3-*O*-glucoside, etc., was also described. Furthermore, analogous to our study, In et al. in their study reported the presence of catechins, epicatechins, kaempferol, and quercetin in quince hydroethanolic peel extract, which further ascertains the presence of the high-value phytoconstituents of pharmacological importance in the quince peel.[24]

2.8. *In Vivo* Cardioprotective Studies of the Best Active Extract. *2.8.1. Electrocardiogram.* The heart dysfunction coherent with the anomalies in electrocardiogram is an important tool used for investigation of cardiotoxicity. DOX administration resulted in considerable variations in the pattern of ECG as evident by heart rate diminution (bradycardia) that is credited to ROS production that in turn disrupts the homeostasis of calcium. The diminution of the calcium present intercellularly results in a decrease in cardiac cell excitability resulting in a decreased heart rate. The heart rate of the control group of the DOX showed a substantial diminution ($p < 0.001$) in contrast to the normal and extract control groups. However, the heart rate in groups pretreated with extract and standards exhibited a substantial rise ($p < 0.01$ (low dose extract group) and $p < 0.001$ (high dose extract group and standard simvastatin and vit E)) in comparison to the control DOX group, suggestive of their

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

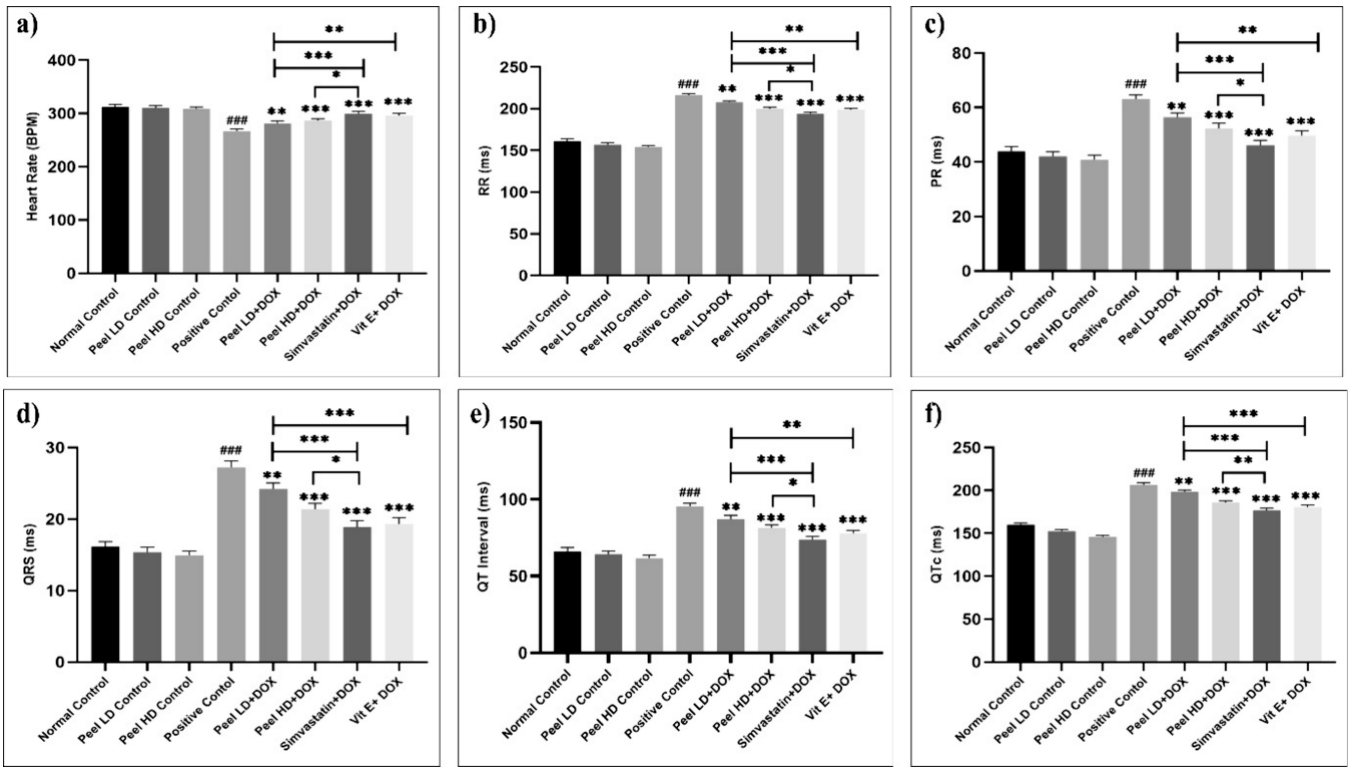


Figure 5. (a) Heart rate (BPM). Statistics are expressed as the (n = 6) mean ± SD. ANOVA (one-way) along with Tukey's multiple comparisons test was used. In comparison to normal control group and extract control groups: $^{###}p < 0.001$; in comparison to DOX control group: $^{***}p < 0.001$, $^{**}p < 0.01$; compared to standard groups $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. (b–f) ECG parameters such as RR, PR, QRS, QT, and QTc interval. Statistics are expressed as (n = 6) mean ± SD. ANOVA (one-way) along with Tukey's multiple comparisons test was used. Compared to normal control group and extract control groups: $^{###}p < 0.001$; in comparison to DOX control group: $^{***}p < 0.001$, $^{**}p < 0.01$; in comparison to standard groups $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

defensive action as shown in Figure 5a. In our study, the finding obtained is in conformity with the findings described by other researchers.[3,35] The group low dose extract + DOX exhibited a substantial diminution ($p < 0.001$ and $p < 0.01$) in the heart rate in contrast to the standard simvastatin and vit E group, respectively. The heart rate in the high dose extract group also showed a significant difference ($p < 0.05$) in contrast to the simvastatin standard group; however, no statistically significant variance was observed in contrast to the vit E group.

A significant alteration in the ECG parameters was seen on DOX administration due to the oxidative stress prompted by DOX. The various ECG parameters, viz., RR, PR, QRS, QT, and QTC interval, demonstrated a significant surge ($p < 0.001$) in contrast to normal and extract control groups. However, the groups pretreated with extracts and standards counteracted these anomalies, conceivably a result of the decrease in the inflammatory markers suggestive of reduction in the cardiomyocyte injury in the pretreated groups that was consistent with the outcomes described by other researchers.[4,36] The ECG parameters are shown in Figure 5b–f. In RR, PR, QRS, QT, and QTC interval, a significant decrease ($p < 0.01$ (low dose extract group) and $p < 0.001$ (high dose extract group and standard simvastatin and vit E group)) was seen in contrast to the control DOX group. The mitigative effect of the standard groups vit E and simvastatin on the RR, PR, QRS, QT, and QTC parameters significantly exceeded that of the low dose of extract ($p < 0.01$–0.001). In case of high dose, a substantial variance ($p < 0.01$–$p < 0.05$) was seen in comparison to the standard simvastatin group. However, no substantial statistical variance ($p > 0.05$) was seen in contrast to the vit E group, conclusive of comparable results of the high dose extract and vit E.

2.8.2. Changes in the Body Weight (Bw), Heart Weight (Hw), and Ratio of Heart Weight to Body Weight (Hw/Bw). The body weight of the rats was noted weekly. The results revealed a steady increase in body weight. However, soon after the induction of DOX, the rats showed an increase in physical exhaustion and reduction in the body weight as shown in Table 4. The necropsy revealed the accretion of an abundant quantity of pericardial, pleural, and peritoneal fluid evocative of the cytotoxicity of DOX as concomitant to the study reported by Xiong et al.[37] The group treated with only DOX displayed an extreme drop in the animal weight (13.13 ± 0.95%) in contrast to the extract and standard pretreated groups: peel LD + DOX (8.5 ± 1.1%) ($p < 0.01$), peel HD+ DOX (7.45 ± 1.20%) ($p < 0.001$), vit E + DOX (6.57 ± 0.97%) ($p < 0.001$), and simvastatin + DOX (5.79 ± 1.0%) ($p < 0.001$).

In contrast to the normal and extract control groups, the DOX group exhibited a reduction ($p < 0.001$) in the weight of the heart. However, groups pretreated with extracts and standards displayed a rise in the weight of heart ($p < 0.001$) in comparison to the control DOX group. Similarly, the heart to body weight ratio (Hw/Bw) in the DOX group was substantially lesser ($p < 0.001$) than the normal and extract control groups. However, in pretreated groups, the Hw/Bw ratio did not differ considerably in contrast to the control DOX group, except for the standard simvastatin group that showed a

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036–40050

Table 4. Measurement of Body Weight (Bw), Heart Weight (Hw), and Heart Weight to Body Weight Ratio (Hw/Bw)[a]

	normal control	peel LD control	peel HD control	positive control	peel LD + DOX	peel HD + DOX	simvastatin + DOX	vit E + DOX
(a) Measurement of body weight (Bw)								
initial BW (g)	85.47 ± 0.69	83.88 ± 0.94	80.34 ± 0.96	73.56 ± 0.43	80.75 ± 0.95	87.43 ± 0.89	70.87 ± 0.77	77.21 ± 0.94
BW in 4 weeks (g)	156.85 ± 1.58	158.35 ± 1.09	158.86 ± 1.07	156.88 ± 1.61	159.14 ± 1.37	162.43 ± 1.70	157.34 ± 1.67	160.21 ± 1.38
final BW (g)	157.29 ± 1.55	158.85 ± 1.92	159.87 ± 1.52	136.27 ± 1.52	145.05 ± 1.59	150.32 ± 1.60	148.22 ± 1.25	149.68 ± 1.76
change in BW (%)	+0.28 ± 0.19	+0.31 ± 0.16	+0.63 ± 0.11	−13.13 ± 0.95	−8.85 ± 1.11**	−7.45 ± 1.20***	−5.79 ± 1.00***	−6.57 ± 0.97***
(b) Measurement of heart weight (Hw) and heart weight to body weight ratio (Hw/Bw)								
Hw (mg)	693.45 ± 5.3	694.01 ± 5.71	699.54 ± 5.67	543.43 ± 4.7###	592.7 ± 4.97***	618.3 ± 4.80***	635.5 ± 5.93***	627.01 ± 5.63***
Hw/Bw (mg/g)	4.40 ± 0.06	4.36 ± 0.10	4.37 ± 0.10	3.98 ± 0.09###	4.08 ± 0.07 ns	4.11 ± 0.06 ns	4.28 ± 0.05**	4.18 ± 0.08ns

[a]Statistics are expressed as (n = 6) mean ± SD. ANOVA (one-way) along with Tukey's multiple comparisons test was used. In comparison to normal control group and extract control groups: ### p < 0.001; in comparison to DOX control group: ***p < 0.001, **p < 0.01, *p < 0.05, ns p > 0.05.

substantial variance (p < 0.01) in contrast to the DOX group. The Hw/Bw ratio was found to decrease in our study, which is ascribed to the loss of myofibrilla and degradation of the myocardial tissue triggered by the cardiotoxic effect of DOX.[38,39]

2.8.3. Serum Biochemical Assays. The measurement of serum biomarkers of cardiac toxicity such as aspartate transaminase, lactate dehydrogenase, and creatinine kinase-MB (AST, LDH, CK-MB) was done to understand the cardiotoxic effect of DOX. The free radical formation as an upshot of the administration of DOX results in the myocyte cell membrane disintegration that in turn causes an increase in the escape of enzymes in blood.[6] Groups pretreated with extracts and standards, however, showed competency to preserve the integrity of the myocardial tissue. The result of the serum biochemical assay is depicted in Figure 6a−c. In our study, a substantial rise (p < 0.001) in the AST, LDH, and CK-MB parameters in the control DOX group was seen in contrast to the normal and extract control groups. The groups pretreated with extracts and standards, however, showed a substantial diminution (p < 0.01 (low dose extract group) and p < 0.001 (high dose extract group and standard simvastatin and vit E)) in the serum parameters in comparison to the control DOX group. The ameliorative outcome of the standard groups vit E and simvastatin on the serum biomarkers (AST, LDH, and CK-MB) was significantly better compared to the low dose of extract (p < 0.01−0.001). In case of high dose, a significant variance (p < 0.05) was seen in contrast to the standard simvastatin group. However, no substantial statistical variance (p > 0.05) was seen in contrast to the vit E group representative of an identical result of high dose of extract and vit E.

DOX is said to alter the metabolism of lipids, thereby resulting in hyperlipidemia by means of inhibition of adipogenesis. It interferes with the biogenesis and breakdown of lipids.[40] However, the pretreated groups significantly improved these serum lipid parameters. The consequence of DOX on the lipid profile is shown in Figure 6d−g. The findings of our study illustrate that in comparison to the normal and extract control groups, the control DOX group displayed a substantial rise (p < 0.001) in the levels of total cholesterol, triglycerides, and low-density lipoprotein (TC, TG, LDL-C), whereas the level of high-density lipoprotein HDL-C considerably declined (p < 0.001). However, the groups pretreated with extracts and standards showed a substantial decrease (p < 0.01 (low dose extract group) and p < 0.001 (high dose extract group and standard simvastatin and vit E)) in the levels of TC, TG, and LDL-C and a significant rise (p < 0.01 (low dose extract group) and p < 0.001 (high dose extract group and standard simvastatin and vit E)) in the level of HDL-C parameters in contrast to the control DOX group. Furthermore, the alleviatory effect of the standard groups vit E and simvastatin on the serum lipid profile (TC, TG, LDL-C, HDL-C) significantly surpassed that of the low dose of extract (p < 0.01−0.001). In case of high dose, a substantial variance (p < 0.05) was found when related to the standard simvastatin group. However, no substantial statistical variance (p > 0.05) was found in contrast to the vit E group, suggestive of a similar result of high dose and vit E except for the LDL-C parameter that showed a significant difference (p < 0.001) with the vit E group also. Mirmohammadlu et al. in their study reported an amelioration of serum parameters in rats (Sprague−Dawley) fed with quince fruit extract that is

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

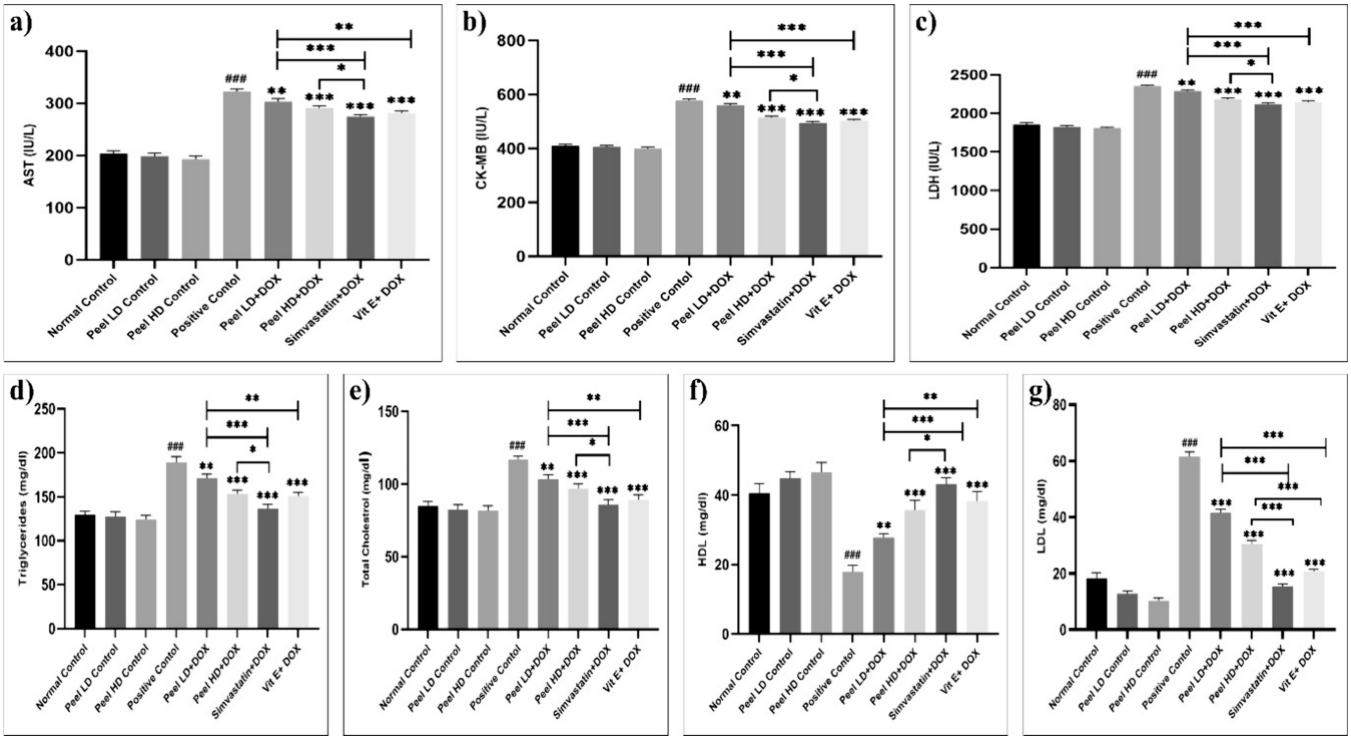


Figure 6. (a) AST (IU/L), (b) CK-MB (IU/L), (c) LDH (IU/L), (d) triglycerides (mg/dL), (e) total cholesterol (mg/dL), (f) HDL-C (mg/dL), and (g) LDL-C (mg/dL). Statistics are expressed as ($n = 6$) mean ± SD. ANOVA (one-way) followed by Tukey's multiple comparisons test was used. In comparison to the normal control group: $^{###}p < 0.001$; in comparison to the DOX control group: $^{***}p < 0.001$, $^{**}p < 0.01$; compared to standard groups: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

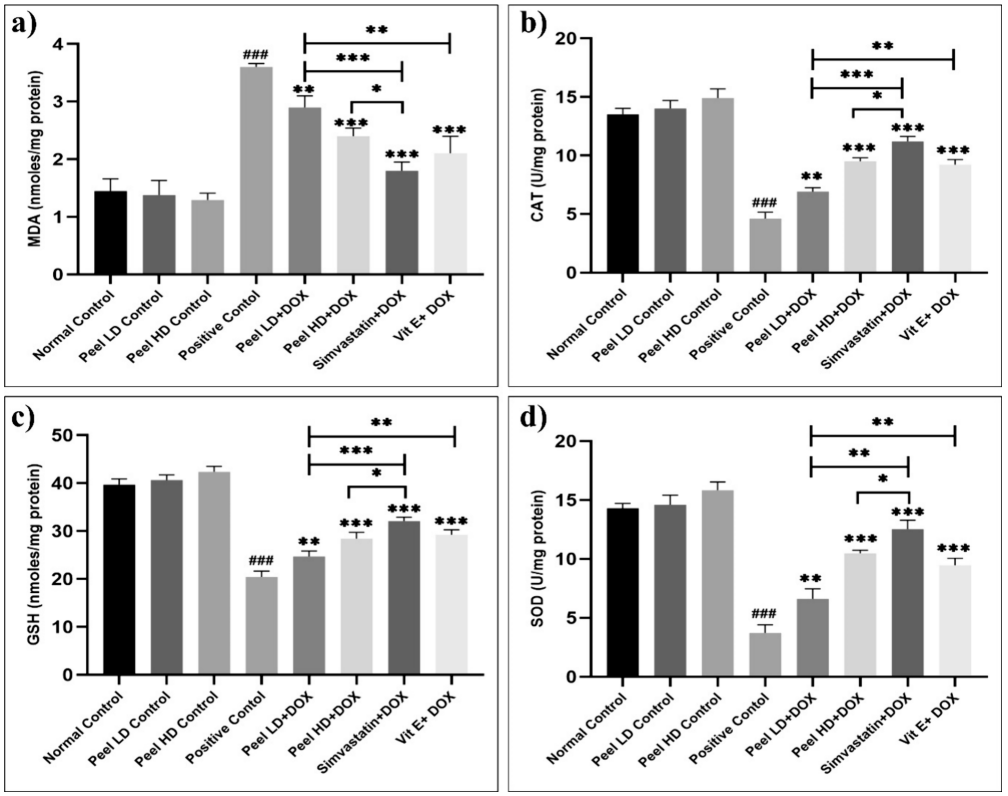


Figure 7. (a) MDA (nmol/mg protein), (b) GSH (nmol/mg protein), (c) SOD (U/mg protein), and (d) CAT (U/mg protein). Statistics ($n = 6$) are expressed as mean ± SD. ANOVA (one-way) along with Tukey's multiple comparisons test was used. In comparison to normal control group: $^{###}p < 0.001$; in comparison to DOX control group: $^{***}p < 0.001$, $^{**}p < 0.01$; in comparison to standard groups $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

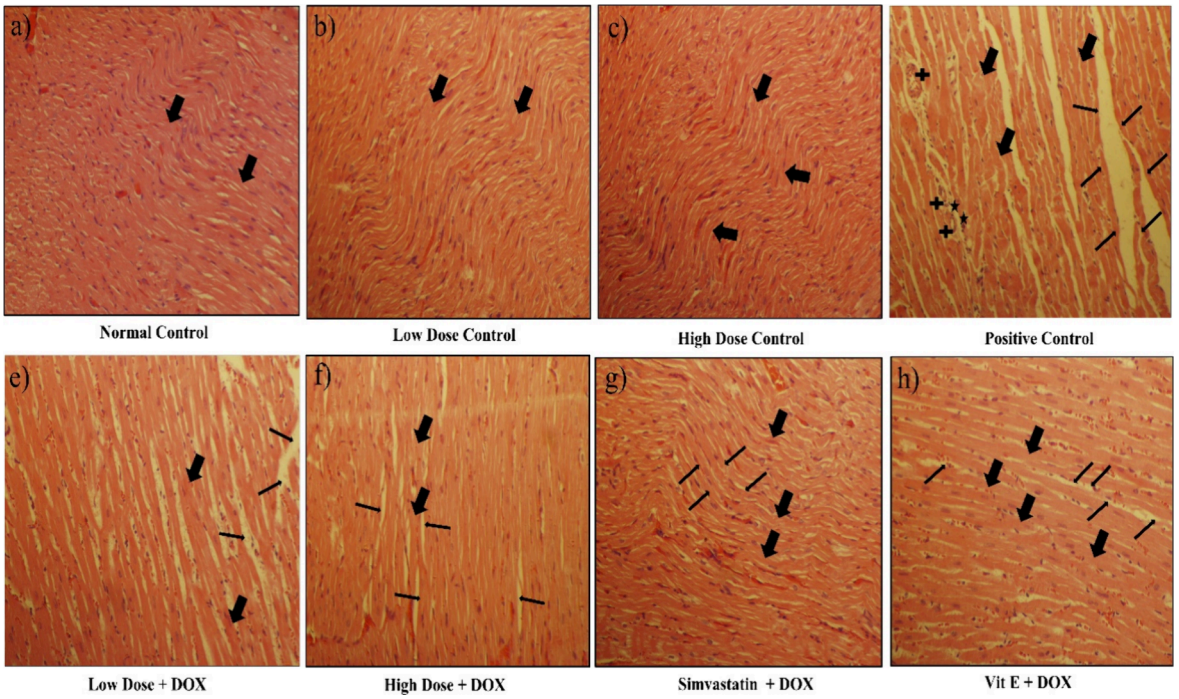


Figure 8. H&E-stained segments of rat heart, observed under high power (160×) of a light microscope. The black arrow (thin) represents cardiomyocyte disintegration, the black arrow (thick) represents cardiac fibers, the star (*) indicates necrosis, and the plus sign (+) indicates vacuolization. (a−c) Normal control rat and extract-fed rats displaying normal myocardial fibers and no vacuolation, necrosis, or inflammation. (d) DOX alone treated rats presenting a big haphazardly shaped hypertrophic myocardial fiber with widespread damage of muscle fibers and occurrence of abundant inflammatory cells. (e) Low dose extract (160 mg/kg p.o.) + DOX-treated rats showing muscle fiber loss and disseminated inflammatory cells in the myocardial fibers. (f) High dose extract (320 mg/kg p.o.) + DOX-treated rats revealing a minor cardiac muscle fiber loss and scarcer disseminated inflammatory cells. (g) Simvastatin (5 mg/kg p.o.) + DOX showing a normal muscle fiber arrangement comparable to the normal. (h) Vit E (100 mg/kg p.o.) + DOX showing slight swelling and muscle fiber injury.

indicative of the hypolipidemic competence of quince, suggesting that quince can efficaciously amend the lipid profile.[41]

2.8.4. Tissue Biochemical Assays. The cardiac tissues undergo oxidative stress upon DOX administration, which in turn causes peroxidation of lipid and henceforth an upsurge in the MDA levels. The subsequent free radical produced causes cell damage that results in the decrease of endogenic cardiac oxidizing compound GSH that otherwise helps maintain the cell integrity.[42] Similarly, the important enzymatic defenses SOD and CAT help preserve the cell structure. A reduction in their level is ascribed to their consumption in the alleviation of oxidative stress.[43] The findings of our study illustrate that the DOX control group displayed a substantial increase in the MDA ($p < 0.001$) in contrast to the normal and extract control groups, which is illustrative of apparent oxidative stress. The groups pretreated with extracts and standards, however, showed a substantial decrease ($p < 0.01$ (low dose extract group) and $p < 0.001$ (high dose extract group and standard simvastatin and vit E)) in the level of MDA in comparison to the control DOX group, suggestive of a suppressive outcome on the lipid peroxidation as shown in Figure 7a. The standard pretreated groups simvastatin and vit E exhibited a substantial reduction ($p < 0.001$ and $p < 0.01$, respectively) in the level of MDA in comparison to the low dose of the extract. The high dose of extract displayed a substantial decrease ($p < 0.05$) in the MDA level in contrast to the standard simvastatin group. However, no statistical variation ($p > 0.05$) was observed in contrast to the standard vit E groups, reminiscent of a comparable outcome of high dose extract and standard vit E. The reactive oxidative species as an outcome of the oxidative stress is arrested by the antioxidants present in the quince, which help reduce the lipid peroxidation and cause deterrence of CVD.[44] Similar results were reported for grape polyphenol extracts that could reverse the doxorubicin-induced cardiomyopathy in Wistar rats[45]

In comparison to the normal and extract control groups, the DOX administration substantially reduced ($p < 0.001$) the level of the GSH, SOD, and CAT enzymes in the control DOX group. The groups pretreated with extract and standards displayed a substantial rise ($p < 0.01$ (low dose extract group) and $p < 0.001$ (high dose extract group and standard simvastatin and vit E)) in the level of GSH, SOD, and CAT in contrast to the control DOX group, corroborative of repressive outcome on cell damage induced by pretreatment as illustrated in Figure 7b−d. In addition, the mitigative effect of the standard groups vit E and simvastatin on the nonenzymatic and enzymatic markers (GSH, SOD, and CAT) was significantly better than the low dose of extract ($p < 0.01$−0.001). In case of high dose, a significant variance ($p < 0.05$) was seen in contrast to the standard simvastatin group. However, no statistically substantial variance ($p > 0.05$) was found in contrast to the vit E group, evocative of comparable results of high dose extract and Vit E standard.

2.8.5. Histopathological Examination of Heart. The outcome of the biochemical tests was ascertained by the histopathological vagaries in the cardiac tissues observed under high power (160×) of a light microscope, as shown in Figure 8. The severity of histopathologic lesions in DOX-treated groups is shown in Table 5 and Figure 9. The normal and the extract

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

Table 5. Severity of Histopathologic Lesions in DOX-Treated Rats[a]

treatment	cardiomyocyte disintegration	vacuolation	necrosis
normal control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
peel LD control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
peel HD control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
positive control	3.00 ± 0.05###	2.30 ± 0.29###	2.78 ± 0.18###
peel LD + DOX	2.46 ± 0.15***	0.00 ± 0.00	1.34 ± 0.19***
peel HD + DOX	1.99 ± 0.20***	0.00 ± 0.00	0.92 ± 0.12***
simvastatin + DOX	1.13 ± 0.14***	0.00 ± 0.00	0.00 ± 0.00
vit E + DOX	1.28 ± 0.17***	0.00 ± 0.00	0.68 ± 0.13***

[a]Statistics are expressed as (n = 6) mean ± SD. ANOVA (one-way) along with Tukey's multiple comparisons test was used. In comparison to normal control group and extract control groups: ### $p < 0.001$; in comparison to DOX control group: ***$p < 0.001$. Grade 1, < 5%; grade 2, 16 ± 25%; grade 3, > 35%.

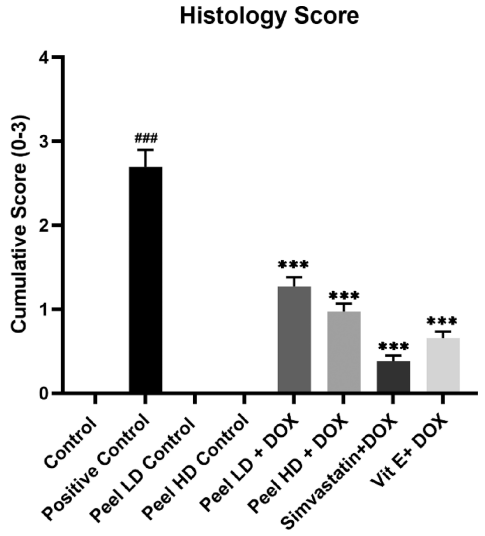


Figure 9. Graph depicting histological scoring. In comparison to normal control group and extract control groups: ### $p < 0.001$; in comparison to DOX control group: ***$p < 0.001$.

control groups displayed usual striated muscles with centrally located nuclei, branched appearance, regular cardiomyocyte, and no necrosis or inflammation (Figure 8a–c). The control groups displayed normal integrity of cardiac fibers and continuity in myofibrils, whereas DOX-treated groups showed disintegration of the myocyte. The control DOX group showed an uncharacteristic alteration in the myocardial tissue. An enormous impairment in cardiac tissue, viz., loss of myofibril, necrosis, vacuolization, and cardiomyocyte disintegration, was seen similar to the results documented by other researchers (Figure 8d).[4,46] However, the groups pretreated with extracts and standards presented slight disintegration in the heart tissue in contrast to the control DOX group, therefore providing a shield to the myocardium (Figure 8e–h). Thus, we could conclude that greater antioxidant and free radical scavenging activity of the quince peel resulted in the recuperation of the myocardium and aided in the attenuation of the cardiac toxicity by means of diminution of the DOX triggered oxidative stress. The quince extract aids in recuperation of the myocardial configuration in rats by impeding the ROS development, lipid peroxidation of cell membrane, and obliteration of mitochondria.[18]

3. CONCLUSIONS

The current research exemplifies the exploration of quince peel as a potent nutraceutical that paves the way in plummeting waste and aids in the accomplishment of the United Nations Sustainable Development Goals 2020. The findings of our study infer quince peels as an important defensive agent against DOX-induced cardiotoxicity. The quince peel extract at a high dose significantly improved the changes caused by DOX in the cardiac tissue. The phytoconstituents present in the peel, as validated by HPTLC and LC–MS, are responsible for the cardioprotective effect. The findings from the biochemical parameters and the histopathological results revealed that quince peel could help reinstate the vagaries concomitant to the DOX-induced toxicity. Overall, it could be concluded that underutilized and undervalued quince peel can be valorized for the extraction of cardioprotective bioactives, which can be used for the functional food product development by the industrial food sector. Its benefits can be foreseen to be reaped for a profitable purpose. However, clinical studies are mandatory for founding its complete efficiency in the assuagement of cardiac disorder.

4. MATERIAL AND METHODS

4.1. Chemicals. All chemicals and reagents used were of analytical grade and procured from SD Fine Chem Pvt. Ltd., Mumbai. Diagnostic kits for analysis were acquired from Nephron Star Healthcare Pvt. Ltd. TLC silica aluminum sheet $60F_{254}$ was from Merck KGaA, 64271 Darmstadt, Germany. Standards (gallic acid, quercetin, and rutin) and drugs doxorubicin (DOX), ketamine, and xylazine were purchased from Sigma-Aldrich, USA. Vitamin E was obtained as a gift sample from Dabur India Ltd., New Delhi. Simvastatin (Sun Pharmaceuticals Ltd.) was acquired from a medical store in New Delhi, India.

4.2. Collection and Preparation of Plant Materials. The quince fruit was sourced from the Horticulture Department orchard (Zakura, Srinagar, J&K) at the time of late September. To eliminate the dirt and other extraneous material, the fruit was subjected to washing with water. The byproduct, i.e., the peel, was subjected to hot air oven dehydration at 45 °C for better retention of bioactive compounds.[47] The dried peel was then pulverized to powder and last sifted via 36 B.S.S. The fine powder obtained was then stockpiled in LDPE pouches and stored in a refrigerator until further use.

4.3. Extraction of Plant Materials. The extraction of quince peel was done using two different methods, viz., heat reflux extraction method and ultrasound assisted extraction method.[48] The extraction was carried out using four different solvents, viz., water or aqueous extract (AE), ethanol or ethanolic extract (EE), methanol or methanolic extract (ME), and water and ethanol in the ratio of 50:50, i.e., hydroethanolic extract (HEE). A 1:10 solid/solvent ratio was taken for both extraction methods. In the case of the heat reflux extraction method, a reflux apparatus was used for extraction. The extraction was carried out for 3 h at 45 °C. For ultrasound

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036–40050

extraction, first, maceration of the sample was done for 24 h, and then the sample was subjected to sonication for 30 min using a bath sonicator (40 kHz, USB-3.5L, PCI Analytics, Maharashtra). The extract filtrate was obtained by passing through Whatman filter paper that was later subjected to drying at 45 °C by means of a rotary vacuum evaporator (KI-102-TS, Khera instruments, New Delhi) and kept at 4 °C for further use.

4.4. Estimation of Total Phenols and Flavonoids. The estimation of total phenol was done using the Folin−Ciocalteau assay.[49,50] Initially, the sample or standard gallic acid (0.5 mL) was mixed with 10% Folin−Ciocalteau's reagent (2.5 mL) and 7.5%, w/v, Na_2CO_3 (2.5 mL). All the contents were than mixed and subjected to incubation at 45 °C for 30 min, and using a spectrophotometer (UV-1601, Shimadzu), reading was noted at 756 nm. The results were specified using gallic acid standard curve in mg of gallic acid per gram equivalent (mg GAE/g).

For flavonoid determination, addition of extract or standard rutin (0.5 mL), 0.1 g/mL $AlCl_3$ (0.1 mL), 1 M CH_3COONa (0.1 mL), and distilled water (2.8 mL) was done, and all the contents were mixed and incubated for a period of 30 min at room temperature. The reading was noted at 415 nm using a spectrophotometer (UV-1601, Shimadzu). The result was stated as milligram of rutin equivalents per gram dry matter of extract (mg RE/g) using a rutin standard curve.[49,50]

4.5. Estimation of Antioxidant Activity. The 2,2-diphenyl-1-picryl-hydrazyl radical activity (DPPH) and reducing power assays were estimated for assessing the antioxidant activity. For the DPPH assay, 0.01 mM DPPH solution was prepared using methanol. Briefly, the sample or standard and DPPH solution, 20 and 180 μL, respectively, were admixed in a 96-well plate that was incubated for 30 min at room temperature away from a light source, and the reading was noted at 517 nm.[51] The DPPH activity was assessed by the following eq 1:

$$\text{DPPH activity}(\%) = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (1)$$

For the reducing power assay, the sample or standard (0.5 mL) was blended with phosphate buffer (2.5 mL, pH 6.6) and 1% potassium ferricyanide (2.5 mL). After thorough mixing of the contents, all samples were incubated for a period of 20 min at room temperature, and subsequently, 10% TCA solution (2.5 mL) was poured in. To this reaction mixture, water (2.5 mL) and 0.1% ferric chloride (0.5 mL) were put together, and the reading was noted @ 700 nm.[52]

4.6. Lipid Peroxidation Assay. The TBARS (thiobarbituric acid-reactive species) method was followed for assessing lipid peroxidation by means of egg yolk mixture (lipid media).[30] Briefly, 10% egg homogenate and extract or standard, 250 and 50 μL, respectively, were put together, and volume makeup to 500 μL was completed using distilled water. $FeSO_4$ (0.07 M, 25 μL) was put in the above mixtures to induce the lipid peroxidation, and the latter was kept for 30 min incubation. Later, 20% acetic acid pH 3.5 (750 μL), (0.8% w/v) TBA (750 μL) prepared in 1.1% sodium dodecyl sulfate (SDS), and 20% TCA (25 μL) were put in, vortexed, and then kept in a boiling water bath for 60 min. The resultant mix was left to cool, 3 mL 1-butanol was put into each tube, and the tubes were centrifugated (3000 rpm, 10 min). The decantation of the top organic layer was done, and absorbance was noted at 532 nm. For the blank sample, 50 μL of the particular solvent was taken instead of the extract sample.

$$\text{Lipid peroxidation inhibition}(\%) = \frac{\text{absorbanceofcontrol} - \text{absorbanceofsample}}{\text{absorbanceofcontrol}} \times 100$$

4.7. FTIR Analysis of the Best Active Extract. The functional groups in the compound were recognized using a Fourier transform infrared spectrophotometer (FTIR). The ATR FTIR was done by using a spectroscope (Bruker Tensor 37, Germany). The sample was loaded, and the scan range and resolution were 400 to 4000 and 4 cm^{-1}, respectively.[53]

4.8. Mineral Analysis of the Best Active Extract. The mineral examination was carried using inductively coupled plasma-mass spectrometry (ICP-MS) by following the AOAC (2005) method. For sample preparation, approximately 1 g of the sample was weighed and subjected to digestion at a temperature of 550 °C in a muffle furnace. The ash of the sample obtained was mixed with HCl, and finally, using milli-Q water, volume makeup was done.

4.9. HPTLC of the Best Active Extract. The high-performance thin layer chromatography (HPTLC) of the best active extract was done for the quantification of rutin. The samples of extract (50 mg/mL) and standard (rutin) were spotted using a Camag Linomat V sample applicator (Switzerland) on a precoated silica aluminum sheet (Merck 60F254) (05 × 10 cm, 0.2 μm thickness). By means of a microliter syringe, bands (5 mm) were formed at a constant application rate (120 nL s^{-1}). The slit dimensions of 5.0 × 0.45 mm and 20 mm s^{-1} scanning speed were used. The HPTLC plates were placed in a solvent saturated glass chamber for 30 min. Linear ascending was allowed to 80 mm. For solvent system optimization, a trial and error method was employed. The solvent system, ethyl acetate/formic acid/acetic acid (11:1.1:1.1), showed the maximum separation of metabolites. The TLC scanner III (Camag) operated by a software (winCats) was used to perform the scanning using wavelengths 254 and 366 nm.[52]

4.10. Metabolite Identification in the Best Active Extract (BAE) by UPLC−MS. Ultraperformance liquid chromatography−mass spectrometry (UPLC−MS) was used for the metabolite profiling of BAE. The BAE was prepared by dissolving in LC−MS-grade methanol passed via a membrane filter (0.2 μm).[52] The Waters ACQUITY UPLC system (serial no. #F09 UPB 920M; model code # UPB; Waters Corp., MA, USA) was used, which was armed with a binary solvent delivery system, autosampler, column manager, and tunable MS detector (Serial No. JAA 272; Synapt; Waters, Manchester, UK). Acetonitrile (A) and water (B) were used as a mobile phase on a monolithic capillary silica-based C18 column (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 100 mm) with a precolumn split ratio of 1:5 min at ambient temperature. Gradient elution mode was used for the separation (initially, 10% A; 0−5 min 40% A; 5−10 min 60% A; 10−13 min, 90% A; 13−15 min, 100% A; 15−16 min 10% A) with a 16 min run time. The nebulizer gas was set to 500L/h, and cone gas was set to 50 L/h. The MS detector had a source temperature of 100 °C. For capillary voltage and cone voltage, values of 3.0 and 40 kV, respectively, were used. For collision, argon gas was used at a pressure of 5.3 × 10^{-5} Torr. For the calculation of

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

mass accurately and for composition of the precursor ions and the fragment ions, the Mass Lynx V 4.1 software (Waters, USA) was used that was installed in the instrument.

4.11. *In Vivo* Cardioprotective Studies of the Best Active Extract. *4.11.1. Animals.* A total of 54 female Wistar rats ($n = 6$), 6 to 8 weeks of age, approximately 70−80 g, were utilized in the study. The sanction from the Institutional Animal Ethics Committee, Jamia Hamdard (registration no. 173/GO/Re/S/2000/CPCSEA, proposal no. 1854) was taken prior to animal procurement. All the animals were kept in a polypropylene cage that was filled with husk. After every 24 h, the husk was changed. The animals were housed at the standard temperature of 20−25 °C, 12 h light/dark cycle, and RH 40−60%. The rats were accustomed a week earlier before the commencement of the experimentation, had nonstop access to water, and were given food pellet *ad libitum*. The experiment was conducted by following the guiding principles of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

4.11.2. Experimental Design. The rats were divided arbitrarily into a group of eight consisting of six rats per group ($n = 6$). The dosages given in the experiment for quince peel, simvastatin, and vitamin E standards were adopted from the literature.[44,54,55] Normal saline was given to group 1 animals, and groups 2 and 3 were fed for 30 days with extract (160 and 320 mg/kg p.o.). The extract was prepared by the reflux extraction method and dried using a rotary vacuum evaporator. The dry extract was then dispersed in distilled water and fed orally to rats via oral gavage. Group 4 animals were administered with DOX (20 mg/kg i.p.) once on the 29th day. The other groups (5, 6, 7, and 8) were given extracts and standards simvastatin (5 mg/kg po) and vit E (100 mg/kg po) for 30 days and DOX on the 29th day.

At the completion of the study on day 31st, euthanization of rats was done using CO_2 inhalation. The blood sample for biochemical estimation was taken via cardiac puncture.[56,57] The serum was separated in a centrifuge (MX-305, AN Tomy, Japan) at 4 °C at 7000 rpm for 15 min.[3] The hearts were removed and suffused with standard ice-cold saline. The heart samples (10% w/v) were subjected to homogenization using a tissue homogenizer in phosphate buffer of pH 7.4. The homogenate was then centrifuged @5000 rpm for 10 min, and supernatant was gathered and kept at −20 °C for further study.[55]

4.11.3. Electrocardiogram. The electrocardiogram (ECG) was recorded by sedating the rats using ketamine and xylazine (60 and 10 mg/kg) by intraperitoneal administration. Briefly, the rats were individually placed on the animal procedure bench, the electrode probes were attached on the hind limbs of the rats, and ECG was recorded.[4] ECG was noted for a total time of 10 min, and quantitative analysis of the data in terms of PR, RR, QT, QTc interval, and QRS complex was done using the software.

4.11.4. Changes in the Body Weight (Bw), Heart Weight (Hw) and Ratio of Heart Weight to Body Weight (Hw/Bw). Body weight variation was noted for a time period of 31 days. On a weekly basis, the weight of rats was taken. On administration of DOX, the percentage change in the body weight was calculated using eq 2.

$$\text{Change in body weight} = \frac{(W_2 - W_1)}{W_1} \times 100 \quad (2)$$

where W_2 = body weight after administration of DOX and W_1 = body weight after 4 weeks.

Later, at the end of the experimentation, animal sacrifice was done, and the weight of the heart and heart to body weight ratio was measured.

4.11.5. Serum Biochemical Assays. Commercial kits were used for the estimation of serum parameters such as aspartate transaminase, lactate dehydrogenase, and creatinine kinase-MB (AST, LDH, CK- MB). The lipid profile parameters such as triglycerides, high-density lipoprotein cholesterol, low density lipoprotein, and total cholesterol (TG, HDL-C, LDL, TC) were also measured by commercially available kits.

4.11.6. Tissue Biochemical Assays. The biochemical assessment of the heart tissue was done by preparing a tissue homogenate (10%) in 0.1 M phosphate buffer with pH 7.4, which was then centrifugated @5000 rpm, and the supernatant was used for assessment of tissue parameters such as malondialdehyde, superoxide dismutase, glutathione, and catalase (MDA, SOD, GSH, CAT).[39,41] Lowry's method was used for protein assessment.[58]

4.11.7. Histopathological Examination of the Heart. Following the animal sacrifice, promptly, the heart was removed and cleaned with chilled standard saline and immediately fixed in 10% formalin solution, which was later entrenched in paraffin. The segmentation and staining for histopathological examination were done using hematoxylin and eosin (H.E.).[55] The sections were examined and characterized on the basis of cardiomyocyte disintegration, vacuolation, and necrosis where 0 = no change, 1 = mild, 2 = moderate, and 3 = severe. The scores signified the following: mild = less than 5% of total myocardium, moderate = 16 ± 25% total myocardium, and severe = more than 35% of total myocardium.[59]

5. STATISTICAL ANALYSIS

The statistical calculation was executed using GraphPad Prism Software, version 8.4.2, by means of one-way ANOVA with Tukey's test. The data were expressed as mean ± standard deviation (SD). The significance at $p < 0.05$ was considered substantial statistically

AUTHOR INFORMATION

Corresponding Authors

Vasudha Sharma − *Department of Food Technology, School of Interdisciplinary Science and Technology, Jamia Hamdard, New Delhi 110062, India*; Email: kotpalvasudha@gmail.com

Farhan J Ahmad − *Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India*; orcid.org/0000-0002-0740-8573; Email: fjahmad@jamiahamdard.ac.in

Authors

Entesar Hanan − *Department of Food Technology, School of Interdisciplinary Science and Technology, Jamia Hamdard, New Delhi 110062, India*

Nazeer Hasan − *Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India*

Sultan Zahiruddin − *Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education & Research, Jamia Hamdard, New Delhi 110062, India*

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050

Sayeed Ahmad − *Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education & Research, Jamia Hamdard, New Delhi 110062, India;* orcid.org/0000-0003-1573-152X

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c00698

Notes

There is no conflict of interest or disclosure associated with the manuscript.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Science and Technology (DST), New Delhi, India, for providing the fellowship to Entesar Hanan (DST/INSPIRE Fellowship/IF170403) and DST-FIST for providing financial assistance to the Department of Food Technology, Jamia Hamdard. The authors would also like to thank Biorender.com for the wonderful illustrations of the graphics.

■ REFERENCES

(1) Piccolo, R.; Giustino, G.; Mehran, R.; Windecker, S. Stable coronary artery disease: Revascularisation and invasive strategies. *Lancet* **2015**, *386* (9994), 702−713.

(2) Kundu, J.; Kundu, S. Cardiovascular disease (CVD) and its associated risk factors among older adults in India: Evidence from LASI Wave 1. *Clin. Epidemiol. Glob. Heal.* **2022**, *13*, 100937.

(3) Warpe, V. S.; Mali, V. R.; Arulmozhi, S.; Bodhankar, S. L.; Mahadik, K. R. Cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in wistar rats. *J. Acute Med.* **2015**, *5* (1), 1−8.

(4) Ahmed, A. Z.; Mumbrekar, K. D.; Satyam, S. M.; Shetty, P.; D'Souza, M. R.; Singh, V. K. Chia Seed Oil Ameliorates Doxorubicin-Induced Cardiotoxicity in Female Wistar Rats: An Electrocardiographic, Biochemical and Histopathological Approach. *Cardiovasc. Toxicol.* **2021**, *21* (7), 533−542.

(5) Rawat, P. S.; Jaiswal, A.; Khurana, A.; Bhatti, J. S.; Navik, U. Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomed. Pharmacother.* **2021**, *139*, 111708.

(6) Akter, H.; et al. Biometabolites of Tamarindus indica play a remarkable cardioprotective role as a functional food in doxorubicin-induced cardiotoxicity models. *J. Funct. Foods* **2022**, *96*, 105212.

(7) Khan, N. et al. Analysis of Major Polyphenolic Compounds of Cydonia oblonga Miller (Quince) Fruit Extract by UPLC-MS/MS and Its Effect on Adipogenesis in 3T3-L1 Cells. *Separations* **2022**, *9* (7), 167.

(8) Srivastava, K. K.; Jabeen, A.; Das, B.; Sharma, A. K. Genetic variability of quince (Cydonia oblonga) in Kashmir valley. *Indian J. Agric. Sci.* **2005**, *75* (11), 766−768.

(9) Hanan, E.; Sharma, V.; Ahmad, F. Nutritional Composition, Phytochemistry and Medicinal Use of Quince (Cydonia oblonga Miller) with Emphasis on its Processed and Fortified Food Products. *J. Food Process. Technol.* **2020**, *11* (6), 1−13.

(10) Ashraf, M. U.; Muhammad, G.; Hussain, M. A.; Bukhari, S. N. A.; Cydonia, O. M. A medicinal plant rich in phytonutrients for pharmaceuticals. *Front. Pharmacol.* **2016**, *7* (JUN),1 DOI: 10.3389/fphar.2016.00163.

(11) El-beltagi, H. S.; Eshak, N. S.; Mohamed, H. I.; Bendary, E. S. A.; Danial, A. W. Physical Characteristics, Mineral Content, and Antioxidant and Antibacterial Activities of Punica granatum or Citrus sinensis Peel Extracts and Their Applications to Improve Cake Quality. *Plants* **2022**, *11* (13), 1740.

(12) Ibrahim, U. K.; Kamarrudin, N.; Suzihaque, M. U. H.; Abd Hashib, S. Local Fruit Wastes as a Potential Source of Natural Antioxidant: An Overview. *IOP Conf. Ser. Mater. Sci. Eng.* **2017**, *206* (1), 012040.

(13) Silva, B. M.; Andrade, P. B.; Valentão, P.; Ferreres, F.; Seabra, R. M.; Ferreira, M. A. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: Antioxidant activity. *J. Agric. Food Chem.* **2004**, *52* (15), 4705−4712.

(14) Silva, B. M.; Andrade, P. B.; Martins, R. C.; Seabra, R. M.; Ferreira, M. A. Principal component analysis as tool of characterization of quince (Cydonia oblonga Miller) jam. *Food Chem.* **2006**, *94* (4), 504−512.

(15) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Study of the organic acids composition of quince (Cydonia oblonga Miller) fruit and jam. *J. Agric. Food Chem.* **2002**, *50* (8), 2313−2317.

(16) Zhang, L. et al. The uhplc-qtof-ms phenolic profiling and activity of cydonia oblonga mill. Reveals a promising nutraceutical potential. *Foods* **2021**, *10* (6), 1230.

(17) Zhou, W.; et al. Effect of Cydonia oblonga Mill. fruit and leaf extracts on blood pressure and blood rheology in renal hypertensive rats. *J. Ethnopharmacol.* **2014**, *152* (3), 464−469.

(18) Fallahi, F.; Roghani, M.; Ansari, I. The effect of hydroalcoholic extract of Cydonia oblonga Miller leaf on doxorubicin-induced cardiac injury in rat. *J. Basic Clin. Pathophysiol.* **2018**, *6* (2), 7−12, DOI: 10.22070/JBCP.2018.3215.1096.

(19) Hanan, E.; Hasan, N.; Zahiruddin, S.; Ahmad, S.; Sharma, V.; Ahmad, F. J. Metabolite profiling and Ameliorative effect of quince (Cydonia oblonga) leaves against doxorubicin induced cardiotoxicity in Wistar rats. *Food Biosci.* **2023**, *53*, 102691.

(20) Amerizadeh, A.; Vaseghi, G.; Esmaeilian, N.; Asgary, S. Cardiovascular Effects of Cydonia oblonga Miller (Quince) Evidence-based Complement. *Altern. Med.* **2022**, *2022*, 1.

(21) Zahiruddin, S.; Parveen, A.; Khan, W.; Parveen, R.; Ahmad, S. TLC-Based Metabolite Profiling and Bioactivity-Based Scientific Validation for Use of Water Extracts in AYUSH Formulations Evidence-based Complement. *Altern. Med.* **2021**, *2021*, 1.

(22) Singh, R. P.; Chidambara Murthy, K. N.; Jayaprakasha, G. K. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. *J. Agric. Food Chem.* **2002**, *50* (1), 81−86.

(23) Safdar, M. N.; Kausar, T.; Jabbar, S.; Mumtaz, A.; Ahad, K.; Saddozai, A. A. Extraction and quantification of polyphenols from kinnow (Citrus reticulate L.) peel using ultrasound and maceration techniques. *J. Food Drug Anal.* **2017**, *25* (3), 488−500.

(24) Othman, S.; Añibarro-Ortega, M.; Dias, M. I.; Ćirić, A.; Mandim, F.; Soković, M.; Ferreira, I. C. F. R.; Pinela, J.; Barros, L.; et al. Valorization of quince peel into functional food ingredients: A path towards ' zero waste ' and sustainable food systems. *Heliyon* **2022**, *8*, e11042.

(25) Alothman, M.; Bhat, R.; Karim, A. A. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem.* **2009**, *115* (3), 785−788.

(26) Adebiyi, O. E.; Olayemi, F. O.; Ning-Hua, T.; Guang-Zhi, Z. In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia. *Beni-Suef Univ. J. Basic Appl. Sci.* **2017**, *6* (1), 10−14.

(27) Do.; et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *J. Food Drug Anal.* **2014**, *22* (3), 296−302.

(28) Dasgupta, N.; De, B. Antioxidant activity of Piper betle L. leaf extract in vitro. *Food Chem.* **2004**, *88* (2), 219−224.

(29) Badmus, J. A.; Adedosu, T. O.; Fatoki, J. O.; Adegbite, V. A.; Adaramoye, O. A.; Odunola, O. A. Lipid peroxidation inhibition and antiradical activities of some leaf fractions of Mangifera indica. *Acta Polym. Pharm. - Drug Res.* **2011**, *68* (1), 23−29.

(30) Upadhyay, R.; Chaurasia, J. K.; Tiwari, K. N.; Singh, K. Antioxidant property of aerial parts and root of phyllanthus fraternus webster, an important medicinal plant. *Sci. World J.* **2014**, *2014*, 1.

(31) Zia, F.; Ghafoor, N.; Iqbal, M.; Mehboob, S. Green synthesis and characterization of silver nanoparticles using Cydonia oblong seed extract. *Appl. Nanosci.* **2016**, *6* (7), 1023−1029.
(32) Wongsa, P.; Phatikulrungsun, P.; Prathumthong, S. FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Sci. Rep.* **2022**, *12* (1), 6631, DOI: 10.1038/s41598-022-10669-z.
(33) Saeed, N. Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.* **2001**, *12* (4), 418−425.
(34) Karar, M. G. E.; Pletzer, D.; Jaiswal, R.; Weingart, H.; Kuhnert, N. Identification, characterization, isolation and activity against Escherichia coli of quince (Cydonia oblonga) fruit polyphenols. *Food Res. Int.* **2014**, *65*, 121−129.
(35) Timm, K. N. et al. Early detection of doxorubicin-induced cardiotoxicity in rats by its cardiac metabolic signature assessed with hyperpolarized MRI. *Commun. Biol.* **2020**, *3* (1) 692, DOI: 10.1038/s42003-020-01440-z.
(36) Patil, P. P. et al. Effect of Theobroma cacao L. on the Efficacy and Toxicity of Doxorubicin in Mice Bearing Ehrlich Ascites Carcinoma. *Antioxidants* **2022**, *11* (6), 1094.
(37) Xiong, C.; et al. Protective effect of berberine on acute cardiomyopathy associated with doxorubicin treatment. *Oncol. Lett.* **2018**, *15* (4), 5721−5729.
(38) Miyoshi, T. et al. LCZ696 ameliorates doxorubicin-induced cardiomyocyte toxicity in rats. *Sci. Rep.* **2022**, *12* (1) 4930, DOI: 10.1038/s41598-022-09094-z.
(39) Shaker, R. A.; Abboud, S. H.; Assad, H. C.; Hadi, N. Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. *BMC Pharmacol. Toxicol.* **2018**, *19* (1)1, DOI: 10.1186/s40360-017-0184-z.
(40) Haybar, H.; et al. Effect of gemfibrozil on cardiotoxicity induced by doxorubicin in male experimental rats. *Biomed. Pharmacother.* **2019**, *109*, 530−535.
(41) Mirmohammadlu, M.; Hosseini, S. H.; Kamalinejad, M.; Gavgani, M. E.; Noubarani, M.; Eskandari, M. R. Hypolipidemic, hepatoprotective and renoprotective effects of Cydonia oblonga Mill. Fruit in streptozotocin-induced diabetic rats. *Iran. J. Pharm. Res.* **2015**, *14* (4), 1207−1214.
(42) Suchal, K. Kampeferol protects against oxidative stress and apoptotic damage in experimental model of isoproterenol-induced cardiac toxicity in rats. *Phytomedicine* **2016**, *23* (12), 1401−1408.
(43) Elblehi, S. S.; El-Sayed, Y. S.; Soliman, M. M.; Shukry, M. Date palm pollen extract avert doxorubicin-induced cardiomyopathy fibrosis and associated oxidative/nitrosative stress, inflammatory cascade, and apoptosis-targeting bax/bcl-2 and caspase-3 signaling pathways. *Animals* **2021**, *11* (3), 1−28.
(44) Umar, A. Effects of Cydonia oblonga Miller leaf and fruit flavonoids on blood lipids and anti-oxydant potential in hyperlipidemia rats. *J. Ethnopharmacol.* **2015**, *169*, 239−243.
(45) Sergazy, S. et al. Cardioprotective effect of grape polyphenol extract against doxorubicin induced cardiotoxicity. *Sci. Rep.* **2020**, *10* (1) 14720, DOI: 10.1038/s41598-020-71827-9.
(46) Hosseini, A. et al. Cardioprotective Effect of Rheum turkestanicum Against Doxorubicin-Induced Toxicity in Rats. *Front. Pharmacol.* **2022**, *13*,909079 DOI: 10.3389/fphar.2022.909079.
(47) Gheisari, H. R.; Khadijeh, A. Drying method effects on the antioxidant activity of quince (Cydonia oblonga miller) tea. *Acta Sci. Polym. Technol. Aliment.* **2014**, *13* (2), 129−134.
(48) Giahi, E.; Jahadi, M.; Khosravi-Darani, K. Enzyme-assisted extraction of glycyrrhizic acid from licorice roots using heat reflux and ultrasound methods. *Biocatal. Agric. Biotechnol.* **2021**, *33*, 101953.
(49) Jan, B.; Zahiruddin, S.; Basist, P.; Irfan, M.; Abass, S.; Ahmad, S. Metabolomic Profiling and Identification of Antioxidant and Antidiabetic Compounds from Leaves of Different Varieties of Morus alba Linn Grown in Kashmir. *ACS Omega* **2022**, *7* (28), 24317−24328.
(50) Khan, M. U.; Zahiruddin, S.; Basist, P.; Krishnan, A.; Parveen, R.; Ahmad, S. Nephroprotective potential of Sharbat-e-Bazoori Motadil (sugar-free) in HEK-293 cells and Wistar rats against cisplatin induced nephrotoxicity. *J. King Saud Univ. Sci.* **2022**, *34* (3), 101839.
(51) Gaurav.; et al. TLC-MS bioautography-based identification of free-radical scavenging, α-amylase, and α-glucosidase inhibitor compounds of antidiabetic tablet BGR-34. *ACS Omega* **2020**, *5* (46), 29688−29697.
(52) Zahiruddin, S. et al. Metabolomic Profiling and Immunomodulatory Activity of a Polyherbal Combination in Cyclophosphamide-Induced Immunosuppressed Mice. *Front. Pharmacol.* **2022**, *12*, 3547 DOI: 10.3389/fphar.2021.647244.
(53) Sharma, V. et al. Characterization of protein extracts from different types of human teeth and insight in biomineralization. *Sci. Rep.* **2019**, *9* (1),9314 DOI: 10.1038/s41598-019-44268-2.
(54) Abliz, A.; et al. Effect of Cydonia oblonga Mill. leaf extract on serum lipids and liver function in a rat model of hyperlipidaemia. *J. Ethnopharmacol.* **2014**, *151* (2), 970−974.
(55) Khan, G.; Haque, S. E.; Anwer, T.; Ahsan, M. N.; Safhi, M. M.; Alam, M. F. Cardioprotective effect of green tea extract on doxorubicin-induced cardiotoxicity in rats. *Acta Polym. Pharm. - Drug Res.* **2014**, *71* (5), 861−868.
(56) Beeton, C.; Garcia, A.; Chandy, K. G. Drawing Blood from Rats through the Saphenous Vein and by Cardiac Puncture. *J. Visualized Exp.* **2007**, no. 7,e266 DOI: 10.3791/266-v.
(57) Parasuraman, S.; Raveendran, R.; Kesavan, R. Blood sample collection in small laboratory animals. *J. Pharmacol. Pharmacother.* **2010**, *1* (2), 87−93.
(58) Schagger, H.; Cramer, W. A.; Vonjagow, G. Protein Measurement With the Folin Phenol Reagent. *Anal. Biochem.* **1994**, *217* (2), 220−230.
(59) Hamza, A. A.; Hassanin, S. O.; Hamza, S.; Abdalla, A.; Amin, A. Polyphenolic-enriched olive leaf extract attenuated doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress and inflammation. *J. Basic Appl. Zool.* **2021**, *82* (1)1, DOI: 10.1186/s41936-021-00251-w.

https://doi.org/10.1021/acsomega.3c00698
ACS Omega 2023, 8, 40036−40050