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Chondroprotection of fruit peels in a monosodium iodoacetate-induced osteoarthritis rat model via downregulation of Col1A1

Nora M. Aborehab[1] | Mahitab H. El Bishbishy[2]

[1]Department of Biochemistry, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt

[2]Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt

Correspondence
Mahitab H. El Bishbishy, Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza 12451, Egypt.
Email: mahelmy@msa.edu.eg

Abstract

The potential of the fruit peels of mango, orange, cantaloupe, and pomegranate in the treatment of osteoarthritis (OA) was evaluated in a rat model. Their metabolic profiles were characterized using ultrahigh-performance liquid chromatography (UPLC)–electrospray ionization-mass spectroscopy and 66 albino rats were intra-articularly injected with monosodium iodoacetate in the knee joints. The extracts were orally administered at doses of 200 and 400 mg/kg for 28 days. Serum levels of IL-6 and tissue levels of cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor-gamma (PPARγ), and alpha-smooth muscle actin (α-SMA) were measured using ELISA. COL1A1 expression was measured by quantitative polymerase chain reaction. Histopathological changes in the joints were examined. In the extracts, 85 metabolites were annotated, and the levels of interleukin (IL)-6, COX-2, α-SMA, malondialdehyde, and nitric oxide were significantly reduced, while PPARγ and glutathione levels were significantly raised in all treated groups compared to the OA group. All extracts downregulated the cartilage mRNA expressions for COL1A1 dose-dependently. Mango peel extract exhibited the best chondroprotective effect. The in silico study showed the link between mango extract metabolites and COX-2.

KEYWORDS
COL1A1, COX-2, fruit peels, osteoarthritis, PPARγ

1 | INTRODUCTION

Fruit peels are considered major agro-industrial by-products and are a good source of polyphenols, carotenoids, vitamins, and other active constituents which are potential therapeutic agents.[1]

As a result of the increased demographic aging and obesity epidemic, osteoarthritis (OA) is becoming more prevalent than in the past few years. Ten percent of American men and 13% of American women who are above 60 years old suffer from symptomatic knee OA. Thus, OA is considered a chief cause of disability and a source of societal cost in the elderly.[2] OA is the most common form of arthritis even more than rheumatoid arthritis, it is a low grade of synovial joint inflammation.[3] It is a complex disease of the whole joint characterized by degradation of cartilage, bone lesions, and

Abbreviations: COL1A1, collagen type 1 alpha1; COX, cyclooxygenase; ESI/MS, electrospray ionization-mass spectroscopy; GSH, glutathione; H&E, hematoxylin and eosin; MDA, malondialdehyde; MIA, monosodium iodoacetate; NO, nitric oxide; PPARγ, peroxisome proliferator-activated receptor gamma; RT-qPCR, reverse transcription quantitative polymerase chain reaction; UPLC, ultrahigh-performance liquid chromatography; α-SMA, alpha-smooth muscle actin.

mixed inflammatory infiltration that appears as severe pain and loss of joint function.[4]

Monosodium Iodoacetate (MIA) induced OA model is well described in rats, especially in terms of the pathological progression of the disease. It triggers the same changes to the histological and pathophysiological features of that in human OA. The mechanism of action of MIA underlying the inhibition of glyceraldehyde-3-phosphate dehydrogenase enzyme which leads to disruption of the glucose metabolism of chondrocytes, reactive oxygen species (ROS) production and caspases activation, and further to the catabolism of cartilage matrix and cell death, that can be detected both in vivo and in vitro. In addition to evoking cartilage degradation, MIA induces an acute inflammation, which is characterized by elevated expression of pro-inflammatory factors such as interleukin (IL)-1β, IL-6, IL-15, cyclooxygenase (COX)-2, and metalloproteinase.[5]

The target for anti-inflammatory drugs is COX, a rate-limiting enzyme involved in the conversion of arachidonic acid into inflammatory prostaglandins. The two isozymes of COX involved in prostaglandin biosynthesis are COX-1 and COX-2. COX-2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as IL-1, TNF-α, lipopolysaccharide.[6] In healthy individuals, COX-2 shows low expression.[7] The computational chemistry has potential implications to understand the mechanism of COX-2 related enzymatic inhibition reactions, and is also applicable in the prediction of more effective inhibitors and engineering 3D structures of other enzymes as well. Therefore, it is interesting to employ the in silico studies to evaluate the COX-2 enzyme inhibition.

Currently, there is no effective pharmacological treatment for OA. As the main causes of the disease remain unknown. The current treatments for OA such as steroids and nonsteroidal anti-inflammatory drugs (NSAIDS) act by reducing joint pain and inflammation. However, their prolonged use had a severe drawback due to their side effects on the gastrointestinal tract and cardiovascular system. Therefore, new alternative treatments with better efficacy and lesser side effects are needed.[8] To our knowledge, a single study reported the evaluation of fruit peel extracts in the treatment of OA, where the passion fruit peel extract was used to reduce knee OA symptoms.[9]

The aim of this study was to characterize the composition of *Mangifera indica* L. (mango), *Citrus sinensis* L. (orange), *Cucumis melo* L. (cantaloupe), and *Punica granatum* L. (pomegranate) fruits peel hydro-ethanolic extracts, evaluate their possible chondroprotective activity in the treatment of OA in the rat model and employ the in silico studies to evaluate the ability of *M. indica* L. fruits peels extract to inhibit COX-2 enzyme. We hypothesize in this study that these fruit peels extracts could perform their chondroprotective activity through anti-inflammatory mechanisms, more specifically COX-2 enzyme inhibition and modulation of COL1A1 expression. This study represents a demonstration of the benefits of agro-industrial by-products as new economical sources of active metabolites.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

2.1.1 | UPLC-ESI/MS characterization of fruit peels extracts

Four different hydro-ethanolic extracts of diverse fruit peels were profiled herein via their UPLC-ESI/MS fingerprints aiming at exploring their compositional metabolites. The chromatograms of all studied extracts in both positive and negative modes were depicted in Figure S1. The identities, observed positive and negative ionization data (molecular and fragment ions), elemental compositions, and relative percentages for individual components are presented in (Table 1).[10–29]

Once the optimal UPLC-ESI/MS conditions had been established for the different extracts, we were able to tentatively assign 85 metabolites. The metabolites were characterized on the basis of their retention times, the molecular mass of parent ion and mass fragmentation behaviors[30] compared to data previously reported in the literature. Given the ionization conditions, a large number of signals were observed due to the advanced fragmentation of the molecules.[31] The metabolites were grouped by their chemical classifications for each extract. Some of them were detected in both ionization modes (positive and negative), while others were only detected in a single mode.

M. indica fruits peels extract was characterized by including five flavonols, four anthocyanins, a benzophenone, a tannin, as well as, two of the characteristic xanthonoids (mangiferin and mangiferin gallate) and also gallic and caffeic acid derivatives. The structures are represented in Figure S2.

Anthocyanins and flavonoids were the major classes of metabolites present in the fruit peel extracts of both *C. melo* and *C. sinensis*. Carotenoids were identified as well, showing characteristic UV-Vis absorbances.[32] In addition to these classes, cucumol A (a triterpenoid characteristic of *C. melo*) and a number of phenolic compounds and acids were also detected in the extract of *C. melo*.

Along with the common classes of metabolites (flavonoids, anthocyanins, and phenolic derivatives), ellagitannins, chalcones, and lignans were identified in *P. granatum* fruits peels extract.

2.2 | Pharmacology/biology

Sixty-six male albino rats were used in this study, which were randomly allocated into 11 groups of six animals each, the effects of different extracts on different biomarkers were as follows.

2.2.1 | Effect of different fruits peels extracts treatment on serum IL-6 level

The mean serum level of IL-6 was significantly increased in the OA group compared to the control group, $p < 0.001$ was observed. While its level was significantly reduced in *M. indica* 200 and 400, *C. sinensis* 400, *C. melo*

TABLE 1 Peak assignment of metabolites in the hydro-ethanolic extract of *Mangifera indica*, *Cucumis melo*, *Citrus sinensis*, and *Punica granatum* fruits peels using liquid chromatography–electrospray ionization-mass spectroscopy in the positive and negative modes

Peak no.	Positive ionization		Negative ionization		Elemental composition	Tentative compound assignment	Relative percentage	Reference
	$[M+H]^+$ (*m/z*)	Product ion fragments (*m/z*)	$[M-H]^-$ (*m/z*)	Product ion fragments (*m/z*)				
***M. indica* fruit peels**								
Flavonoids–flavonols								
1	303.3	275.1, 245.3, 181.4	n.d.	n.d.	$C_{15}H_{10}O_7$	Quercetin	0.65	[11]
2	465.1	303.1	463.3	301	$C_{21}H_{20}O_{12}$	Quercetin-7-*O*-glucoside	0.31	[15]
3	465.3	303.1, 302.1, 270.1, 181.4	463.2	301, 300.2, 267.5, 179.1	$C_{21}H_{20}O_{12}$	Querecetin-3-*O*-glucoside	6.77	[12]
4	449.1	303.1, 181.4, 153	n.d.	n.d.	$C_{21}H_{20}O_{11}$	Quercetin-3-*O*-rhamnoside	0.58	[16]
5	n.d.	n.d.	625.3	301.1, 300.2	$C_{27}H_{30}O_{17}$	Quercetin-3-*O*-glucosyl glucoside	1.2	[15]
Anthocyanins								
6	463.3	303.1	n.d.	n.d.	$C_{22}H_{23}O_{11}$	Peonidin-3-*O*-glucoside	0.05	[15]
7	n.d.	n.d.	546.9	301.2	$C_{25}H_{25}O_{14}$	Peonidin-3-*O*-malonyl glucoside	4.89	[15]
8	n.d.	n.d.	533.2	331.4	$C_{25}H_{27}O_{13}$	Malvidin-3-*O*-acetyl glucoside	1.52	[15]
9	n.d.	n.d.	574.9	423.4, 284.5, 193.2	$C_{30}H_{24}O_{12}$	Procyanidin A	12.25	[16]
Xanthonoids								
10	423.1	333, 303.3, 273.1	421.0	331, 301.2, 271.3, 183	$C_{19}H_{18}O_{11}$	Mangiferin	23.68	[17]
11	575.4	423.1, 303.1	573.1	421, 301.1	$C_{26}H_{22}O_{15}$	Mangiferin gallate	3.88	[18]
Benzophenones								
12	425.3	335.2, 305, 195.1, 170.8	n.d.	n.d.	$C_{19}H_{20}O_{12}$	Maclurin-3-*C*-glucoside	2.39	[17]
Tannins								
13	471.1	435.1, 303.3	469.1	433.3, 301.1	$C_{21}H_{10}O_{13}$	Valoneic acid dilactone	0.22	[17]
Gallic and caffeic acid derivatives								
14	171.0	127.3	n.d.	n.d.	$C_7H_6O_5$	Gallic acid	0.79	[13]
15	n.d.	n.d.	342.9	192.1, 91	$C_{14}H_{16}O_{10}$	Theogallin	2.91	[17]

(Continues)

TABLE 1 (Continued)

Peak no.	Positive ionization $[M+H]^+$ (*m/z*)	Positive ionization Product ion fragments (*m/z*)	Negative ionization $[M-H]^-$ (*m/z*)	Negative ionization Product ion fragments (*m/z*)	Elemental composition	Tentative compound assignment	Relative percentage	Reference
16	n.d.	n.d.	197.0	125.1, 124.2	$C_9H_{10}O_5$	Ethyl gallate	4.65	[17]
17	502.8	457, 304.9, 198.8	501.1	455.2, 303.3, 197.2	$C_{23}H_{18}O_{13}$	Ethyl *p*-trigallate	4.03	[17]
18	485.4	171.3, 127.3	n.d.	n.d.	$C_{20}H_{20}O_{14}$	Digalloyl glucose	7.26	[17]
C. *melo* fruit peels								
Anthocyanins								
1	478.7	316.8	n.d.	n.d.	$C_{22}H_{23}O_{12}$	Petunidin-3-*O*-glucoside	0.07	[19]
2	463	332.6	n.d.	n.d.	$C_{22}H_{23}O_{11+}$	Malvidin-3-*O*-arabinoside	0.21	[20]
3	449.1	287.4	n.d.	n.d.	$C_{21}H_{21}O_{11+}$	Cyanidin-3-*O*-glucoside	10.94	[19]
4	595.1	449.3	593.2	447.4	$C_{27}H_{31}O_{15+}$	Cyanidin-3-*O*-rutinoside	0.12	[21]
5	419.1	287.4	n.d.	n.d.	$C_{20}H_{19}O_{10}$	Cyanidin-3-*O*-arabinoside	0.47	[20]
Carotenoids								
6	537	444.3, 387.6	n.d.	n.d.	$C_{40}H_{56}$	α-Carotene	2.23	[10]
7	569.1	476.4, 459.3, 337.7	n.d.	n.d.	$C_{40}H_{56}O_2$	Lutein	0.11	[10]
8	551.8	521.2	n.d.	n.d.	$C_{40}H_{56}O$	β-Cryptoxanthin	0.05	[10]
Flavonoids–flavanones								
9	435.4	273.2, 155.1	433.2	271.1, 153.4	$C_{21}H_{22}O_{10}$	Naringenin-7-*O*-glucoside	0.04	[22]
10	303.1	260.4, 144.7	300.8	257.8, 143.1	$C_{16}H_{14}O_6$	Hesperetin	10.62	[22]
11	610.7	303.1	n.d.	n.d.	$C_{28}H_{34}O_{15}$	Hesperetin-7-*O*-rutinoside (Hesperidin)	0.17	[13]
Flavonoids–flavonols								
12	n.d.	n.d.	269.4	227.1	$C_{15}H_{10}O_5$	Galangin	2.81	[22]
13	319.1	181.2	317.3	179.1	$C_{15}H_{10}O_8$	Myricetin	0.19	[22]
14	465.2	318.2	463.1	316.3	$C_{21}H_{20}O_{12}$	Myricetin-3-*O*-rhamnoside (myricitrin)	0.04	[22]
15	481.2	319.1, 181.3	478.7	316.8, 179.1	$C_{21}H_{20}O_{13}$	Myricetin-3-*O*-glucoside	0.12	[22]
Flavonoids–flavones								
16	n.d.	n.d.	252.7	181.2, 151	$C_{15}H_{10}O_4$	5,7-dihydroxyflavone (chrysin)	3.15	[14]

TABLE 1 (Continued)

Peak no.	Positive ionization $[M+H]^+$ (*m/z*)	Product ion fragments (*m/z*)	Negative ionization $[M-H]^-$ (*m/z*)	Product ion fragments (*m/z*)	Elemental composition	Tentative compound assignment	Relative percentage	Reference
Triterpenoids								
17	443.1	443.1	n.d.	n.d.	$C_{30}H_{50}O_2$	Cucumol A	19.47	[23]
Phenolic compounds and acids								
18	n.d.	n.d.	311.1	148.8, 179.1, 135.2	$C_{13}H_{12}O_9$	Caftaric acid	0.36	[22]
19	485.2	314.6, 171.2	483.1	313.2, 168.7	$C_{20}H_{20}O_{14}$	1,6-Di-*O*-galloyl glucoside	0.17	[22]
20	n.d.	n.d.	183.0	169.2, 125.3	$C_8H_8O_5$	Methyl gallate	2.12	[22]
21	343.2	181	341.2	178.7, 135.2	$C_{15}H_{18}O_9$	Caffeoyl glucose	0.03	[22]
22	369.3	175.1, 193.3	367.2	173.2, 191.3	$C_{17}H_{20}O_9$	4-Ferolyl quinic acid	12.28	[22]
23	n.d.	n.d.	175.2	115.4	$C_6H_8O_6$	Ascorbic acid	1.24	[22]
C. *sinensis* fruit peels								
Anthocyanins								
1	n.d.	n.d.	447.1	317.3	$C_{22}H_{23}O_{12}$	Petunidin-3-*O*-arabinoside	3.01	[20]
2	463	332.6	461.1	331.2	$C_{22}H_{23}O_{11}+$	Malvidin-3-*O*-arabinoside	0.28	[20]
3	n.d.	n.d.	461.1	301.2	$C_{22}H_{23}O_{11}+$	Peonidin-3-*O*-pyranoside	2.11	[20]
Carotenoids								
4	537	444.3, 387.6	535.1	398.2, 411.1	$C_{40}H_{56}$	α-Carotene	9.55	[10]
5	569.1	476.4, 459.3, 337.7	n.d.	n.d.	$C_{40}H_{56}O_2$	Lutein	4.8	[10]
6	551.8	521.2	n.d.	n.d.	$C_{40}H_{56}O$	β-Cryptoxanthin	0.29	[10]
7	n.d.	n.d.	595.1	595.1	$C_{40}H_{52}O_4$	Astaxanthin	1.91	[10]
Flavonoids–flavanones								
8	581.2	383.4, 272.8	579.2	459, 271.1	$C_{21}H_{22}O_{10}$	Naringenin-7-*O*-neohesperidoside (naringin)	5.38	[12]
9	303.1	260.4, 144.7	300.8	257.8, 143.1	$C_{16}H_{14}O_6$	Hesperetin	4.69	[24]
10	610.7	303.1	n.d.	n.d.	$C_{28}H_{34}O_{15}$	Hesperetin-7-*O*-rutinoside (hesperidin)	13.08	[13]

(Continues)

TABLE 1 (Continued)

Peak no.	Positive ionization $[M+H]^+$ (*m/z*)	Product ion fragments (*m/z*)	Negative ionization $[M-H]^-$ (*m/z*)	Product ion fragments (*m/z*)	Elemental composition	Tentative compound assignment	Relative percentage	Reference
Flavonoids–flavones								
11	n.d.	n.d.	447.1	357.1, 327, 299.1	$C_{21}H_{20}O_{11}$	Luteolin-8-*C*-glucoside (orientin)	0.04	[12]
12	595.1	475.3, 355.4	593.1	512.2, 473.2, 353.2	$C_{27}H_{30}O_{15}$	Apigenin-6,8-*C*-diglucoside (vicenin 2)	4.75	[12]
13	n.d.	n.d.	431.1	340.7, 311.1, 283.2	$C_{21}H_{20}O_{10}$	Apigenin 8-*C*-glucoside (vitexin)	0.11	[12]
14	n.d.	n.d.	431.4	413.1, 311.1, 283.2	$C_{21}H_{20}O_{10}$	Apigenin 6-*C*-glucoside (isovitexin)	0.17	[12]
15	579.1	402.9, 295.1, 270.8	n.d.	n.d.	$C_{27}H_{30}O_{14}$	Apigenin 7-*O*-neohesperidoside (rhoifolin)	0.07	[12]
16	n.d.	n.d.	268.2	268.2	$C_{15}H_{10}O_5$	Apigenin	1.25	[13]
17	n.d.	n.d.	371.2	356.1, 341	$C_{20}H_{20}O_7$	Tangeretin	0.28	[25]
18	609.1	301.3, 565.4, 286.4	n.d.	n.d.	$C_{28}H_{32}O_{15}$	Diosmin	0.24	[24]
19	609.1	301.3, 565.4, 286.4	n.d.	n.d.	$C_{28}H_{32}O_{15}$	Neodiosmin	0.12	[26]
20	n.d.	n.d.	623.2	503.4, 383.2, 299.3	$C_{28}H_{32}O_{16}$	Chrysoeriol 6,8-di-*C*-glucoside (stellarin 2)	0.05	[26]
21	373.4	358, 297.1	n.d.	n.d.	$C_{20}H_{20}O_7$	Sinensetin	4.8	[25]
22	403	355.1, 327.4	n.d.	n.d.	$C_{21}H_{22}O_8$	Nobiletin	1.79	[25]
Flavonoids–flavonols								
23	611.3	303.3	609.2	427.1, 301.3, 271.2	$C_{27}H_{30}O_{16}$	Quercetin-3-*O*-rutinoside (rutin)	9.55	[12]
24	465.3	303.3	463.4	301.3, 179.4	$C_{21}H_{20}O_{12}$	Quercetin-3-*O*-glucoside (isoquercitrin)	0.66	[12]
***P. granatum* fruit peels**								
Ellagitannins								
1	1084.7	783.2, 603.4	n.d.	n.d.	$C_{48}H_{28}O_{30}$	Punicalagin α	0.16	[27]
2	1084.7	783.2, 603.4	n.d.	n.d.	$C_{48}H_{28}O_{30}$	Punicalagin β	0.2	[27]
3	n.d.	n.d.	951.2	933.1, 914.5, 301.3	$C_{41}H_{28}O_{27}$	Galloyl-HHDP-DHHDP-hex (granatin B)	0.25	[28]

TABLE 1 (Continued)

Peak no.	Positive ionization $[M+H]^+$ (*m/z*)	Positive ionization Product ion fragments (*m/z*)	Negative ionization $[M-H]^-$ (*m/z*)	Negative ionization Product ion fragments (*m/z*)	Elemental composition	Tentative compound assignment	Relative percentage	Reference
4	785.2	767.1, 483.4, 302.8	n.d.	n.d.	$C_{34}H_{24}O_{22}$	Pedunculagin I isomer	15.47	[27]
5	n.d.	n.d.	783.3	765.2, 301.3	$C_{34}H_{24}O_{22}$	Bis-HHDP-hex (pedunculagin II)	28.34	[28]
Chalcones								
6	436.8	275.2	435.2	272.9	$C_{21}H_{24}O_{10}$	Phloretin-hexoside (phlorizin)	0.7	[27]
7	275.1	168.7	n.d.	n.d.	$C_{15}H_{14}O_5$	Phloretin	0.14	[27]
Lignans								
8	519.2	338.7	n.d.	n.d.	$C_{26}H_{29}O_{11}$	Feruloyl coniferin	17.44	[27]
9	358.6	328.1, 152.9, 138.4	n.d.	n.d.	$C_{20}H_{22}O_6$	Pinoresinol	2.32	[27]
10	523.4	361.3, 346.1	n.d.	n.d.	$C_{26}H_{36}O_{12}$	Cyclolariciresinol hexoside	0.06	[27]
Flavonoids								
11	595.3	287	593.2	284.5	$C_{27}H_{30}O_{15}$	Kaempferol rutinoside	3.35	[27]
12	450.8	289.2, 271.1, 261.4	n.d.	n.d.	$C_{21}H_{22}O_{11}$	Dihydrokaempferol hexoside	0.03	[27]
13	307.4	181.4	305.2	179.1	$C_{15}H_{14}O_7$	(+)-Gallocatechin	13.18	[27]
Anthocyanins								
14	611.1	449.3, 287.2	608.7	447.1, 284.5	$C_{27}H_{31}O_{16}$	Cyanidin-3,5-diglucoside	2.73	[29]
15	n.d.	n.d.	463.3	301.3	$C_{21}H_{21}ClO_{12}$	Delphinidin-3-glucoside	0.48	[29]
16	627.1	465.3, 302.8	n.d.	n.d.	$C_{27}H_{31}O_{17}$+	Delphinidin-3,5-diglucoside	0.09	[29]
17	449.1	287.4	n.d.	n.d.	$C_{21}H_{21}O_{11}$+	Cyanidin-3-*O*-glucoside	1.36	[28]
Acids and derivatives								
18	170.6	170.6	169	169	$C_7H_6O_5$	Gallic acid	0.1	[13]
19	193.1	175.1, 113.3	n.d.	n.d.	$C_6H_8O_7$	Citric acid	3.08	[27]
20	300.7	241.1, 181.4, 139.2	n.d.	n.d.	$C_{13}H_{16}O_8$	Hydroxybenzoic acid hexoside	0.04	[27]

TABLE 2 Effect of different fruits peels extracts treatment on serum interleukin-6 (IL-6), tissue cyclooxygenase-2 (Cox-2), tissue peroxisome proliferator-activated receptor gamma (PPARγ), and tissue alpha-smooth muscle actin (α-SMA) levels

Groups	IL-6 (pg/ml)	Cox-2 (ng/ml/1 g total protein)	PPARγ (ng/ml/1 g total protein)	α-SMA (ng/ml/1 g total protein)
Control	242 ± 29.1	5.5 ± 1.9	5.63 ± 0.38	1.71 ± 0.43
OA	379 ± 64[a]	12.9 ± 2.23[a]	4.37 ± 0.52[a]	5.04 ± 1.73[a]
Mangifera indica 200	290 ± 51.9[b]	8.76 ± 1.47[a,b]	4.61 ± 0.44[a]	2.28 ± 0.31[a,b]
M. indica 400	236 ± 27.6[b]	5.74 ± 1.1[b]	5.6 ± 0.17[b]	1.61 ± 0.27[b]
Citrus sinensis 200	356 ± 35.4[a]	10.01 ± 0.77[a,b]	4.3 ± 0.49[a]	4.39 ± 1.32[a]
C. sinensis 400	278 ± 15.5[a,b,c]	9.14 ± 0.52[a,b,c]	5.84 ± 0.52[b]	2.94 ± 0.61[b,c]
Cucumis melo 200	315 ± 7.73[a,b]	9.83 ± 1.36[a,c]	4.81 ± 0.29[a]	2.98 ± 0.31[a,b]
C. melo 400	282 ± 24.1[a,b,c]	7.72 ± 1.63[b]	5.6 ± 0.37[b]	1.68 ± 0.56[b]
P. granatum 200	354 ± 47.3[a]	12.5 ± 2.47[a]	4.13 ± 0.41[a]	2.88 ± 0.49[a,b]
P. granatum 400	285 ± 40.1[a,b,c]	7.5 ± 2.15[b]	5.52 ± 0.60[b]	1.56 ± 0.67[b]
Voltaren 30	234 ± 20.7[b]	5.77 ± 1.03[b]	5.57 ± 0.81[b]	1.63 ± 0.31[b]

Note: Results were expressed as mean ± SD and analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test.
[a]Significant from control at $p < 0.001$.
[b]Significant from OA group at $p < 0.01$.
[c]Cignificant from *M. indica* 400 group at $p < 0.01$.

200 and 400, and *P. granatum* 400 groups compared to the OA group ($p < 0.01$). Also, the mean serum level of IL-6 was significantly reduced in *M. indica* 400 group compared to *C. sinensis* 400, *C. melo* 400, and *P. granatum* 400 groups ($p < 0.01$). No statistically significant difference was observed in the level of IL-6 between the control group and *M. indica* 200 and 400 and Voltaren 30 groups (Table 2 and Figure S3).

2.2.2 | Effect of different fruits peels extracts treatment on tissue COX-2 level

The mean level of COX-2 was significantly increased in the OA group compared to the control group $p < 0.001$, while a significant reduction was observed in *M. indica* 200 and 400, *C. sinensis* 200 and 400, *C. melo* 200 and *C. melo* 400, and *P. granatum* 400 groups when compared to OA group at $p < 0.01$. Also, *M. indica* 400 and *P. granatum* 400 groups showed the best effect in decreasing the level of COX-2 than the other peels extracts at the same dose level similar to the Voltaren effect (Table 2 and Figure S4).

2.2.3 | Effect of different fruits peels extracts treatment on tissue peroxisome proliferator-activated receptor gamma (PPARγ) level

A significant reduction in the level of PPARγ in cartilage tissue in OA group compared to control group, *p*-value was < 0.001, on the other hand, its level was significantly raised in *M. indica* 400, *C. sinensis* 400, *C. melo* 400, *P. granatum* 400, and Voltaren groups compared to OA group ($p < 0.01$). Also, no statistically significant difference was observed in the level of PPARγ between control group and *M. indica* 400, *C. sinensis* 400, *C. melo* 400, *P. granatum* 400, and Voltaren groups (Table 2 and Figure S5).

2.2.4 | Effect of different peels extracts treatment on tissue alpha-smooth muscle actin (α-SMA) level

The mean level of α-SMA was significantly increased in the OA group compared to the control group $p < 0.001$, while a significant reduction was observed in *M. indica* 200 and 400, *C. sinensis* 400, *C. melo* 200 and 400, *P. granatum* 200 and 400, and Voltaren 30 groups when compared to OA group at $p < 0.01$. Also, no statistically significant difference was found between *M. indica* 400, *C. melo* 400, and *P. granatum* 400 groups in reducing the level of α-SMA which was similar to the Voltaren effect (Table 2 and Figure S6).

2.2.5 | Effect of different fruits peels extracts treatment on tissue malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) levels

The mean level of MDA and NO were significantly increased in the OA group compared to the control group $p < .001$, while a significant reduction was observed in *M. indica* 200 and 400, *C. sinensis* 200 and 400, *C. melo* 200 and 400, *P. granatum* 200 and 400, and Voltaren 30 groups when compared to OA group at $p < 0.01$. On the other hand, a significant reduction in NO level was observed in *M. indica* 200 and 400, *C. sinensis* 400, *C. melo* 400, *P. granatum* 400, and Voltaren 30 groups when compared to the OA group at $p < 0.01$. Also, a significant difference was

TABLE 3 Effect of different peels extracts treatment on tissue malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) levels

Groups	MDA (nmol/ml/g total protein)	NO (µmol/L/g total protein)	GSH (nmol/dl/g total protein)
Control	7.18 ± 0.48	14.7 ± 1.28	2.02 ± 0.30
OA	11.4 ± 1.49[a]	21.7 ± 2.66[a]	1.58 ± 0.08[a]
Mangifera indica 200	8.26 ± 1.22[b]	18.2 ± 1.04[a,b]	1.62 ± 0.25[a]
M. indica 400	7.28 ± 0.66[b]	15.1 ± 0.88[b]	2.24 ± 0.09[b]
Citrus sinensis 200	9.28 ± 1.12[a,b]	20.4 ± 1.97[a]	1.29 ± 0.34[a]
C. sinensis 400	8.2 ± 0.45[b,c]	17.6 ± 0.74[b,c]	1.80 ± 0.12[c]
Cucumis melo 200	8.81 ± 0.47[b]	20.8 ± 2.5[a]	1.46 ± 0.18[a]
C. melo 400	7.85 ± 0.84[b]	18.2 ± 1.54[b,c]	1.90 ± 0.29[c]
Punica granatum 200	9.63 ± 1.11[a,b]	20.8 ± 2.01[a]	1.07 ± 0.28[a]
P. granatum 400	8.93 ± 1.41[b,c]	18.3 ± 2.11[a,b,c]	1.50 ± 0.39[c]
Voltaren 30	6.78 ± 0.29[b]	14.8 ± 0.96[b]	2.34 ± 0.19[b]

Note: Results were expressed as mean ± SD and analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test.
[a]Significant from control at $p < 0.001$.
[b]Significant from OA group at $p < 0.01$.
[c]Significant from *M. indica* 400 group at $p < 0.01$.

found between *M. indica* 400, *C. sinensis* 400, and *P. granatum* 400 groups ($p < 0.01$) in reducing the level of MDA which was similar to the Voltaren effect. *M. indica* 400 showed the best effect in reducing NO level compared to other peels extracts similar to the Voltaren effect (Table 3).

GSH level was significantly reduced in the OA group compared to the control group, $p < 0.001$, also a significant increase was observed in *M. indica* 400 and Voltaren 30 groups compared to OA group at $p < 0.01$, finally *M. indica* 400 showed a significant increase in GSH level compared to other peels extracts which was similar to the Voltaren effect (Table 3).

2.2.6 | Effect of different peels extracts treatment on mRNA expression of collagen, type I, alpha1 (COL1A1)

The *M. indica*, *C. sinensis*, *C. melo*, and *P. granatum* treatments dose-dependently downregulated the rats' cartilage mRNA expressions for COL1A1 (the higher dose showed better effects than the lower dose). Also, the Voltaren treatment showed the same effect as *M. indica* 400. Additionally, the *M. indica* at the high dose (400 mg/kg) showed a prominent effect over all other peels extracts (Figure 1).

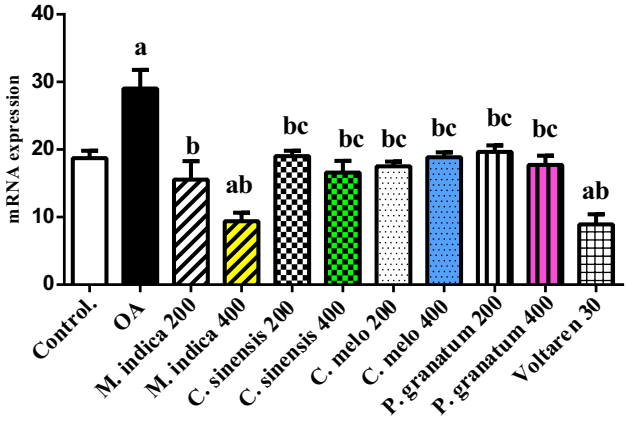

FIGURE 1 mRNA expression of COLIA1 after 28 days of treatment with the different peels extracts. Results were expressed as mean ± SD and analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test, a: significant from control at $p < 0.001$, b: significant from OA group at $p < 0.01$, c: significant from *Mangifera indica* 400 groups at $p < 0.01$

2.2.7 | Histopathological changes associated with fruit peels extract treatment

Sections from the control group (C) showed normal histological appearance and proteoglycan contents of the cartilage as they showed preserved integrity of the articular surface, normal orientation, and distribution of chondrocytes, and preservation of superficial layer. The OA group showed histological features recognized by thinning out of the cartilage with abnormal changes (clefting, calcification, fibrosis, and matrix changes). All other groups showed different grades of OA illustrated in Figure 2.

2.3 | Molecular docking

2.3.1 | Molecular docking of *M. indica* fruit peels metabolites

In molecular modeling studies, the more similar the docking overlap between the drugs with its receptor, the lower the energy that is

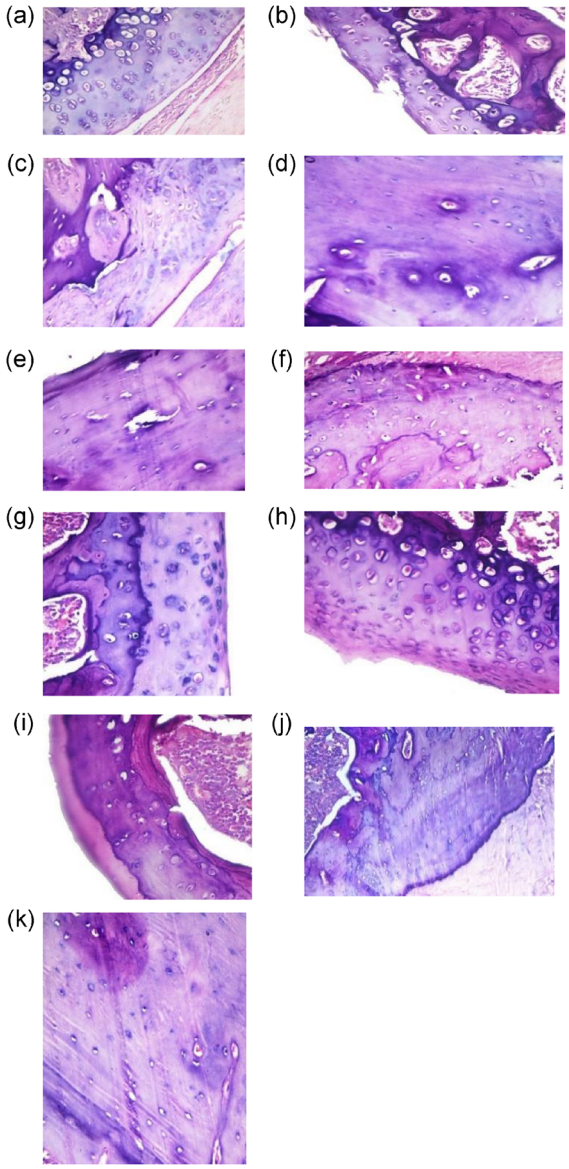

FIGURE 2 Histopathological sections of (a) control group with an average thickness of articular cartilage and normal chondrocytes orientation. (b) Osteoarthritic group showing thinning of cartilage, clefting, abnormal orientation, fissures, and cracking. (c) *Punica granatum* 200 mg/kg showing improvement in the thickness of cartilage but still, erosion and clefting are present. (d) *P. granatum* 400 mg/kg with more improvement in the thickness than *P. granatum* 200 mg/kg. (e) *Cucumis melo* 200 mg/kg with improvement in thickness but still cracking present. (f) *C. melo* 400 mg/kg with mild improvement in cracking than *C. melo* 200 mg/kg. (g) *Citrus sinensis* 200 mg/kg with average thickness of cartilage, no cracks, no erosions and no fissures. (h) *C. sinensis* 400 mg/kg with improvement in thickness of cartilage than *C. sinensis* 200 mg/kg, no cracks, no erosions, and no fissures. (I) *M. indica* 200 mg/kg with average thickness of cartilage, better density of matrix, no fissures, no cracks, and no erosion. (j) *M. indica* 400 mg/kg with better thickness in cartilage than *M. indica* 200 mg/kg, no fissures, no cracks, and no erosion. (k) Voltaren 30 mg/kg with average thickness in cartilage and minor cracks and fissures. Magnification ×400

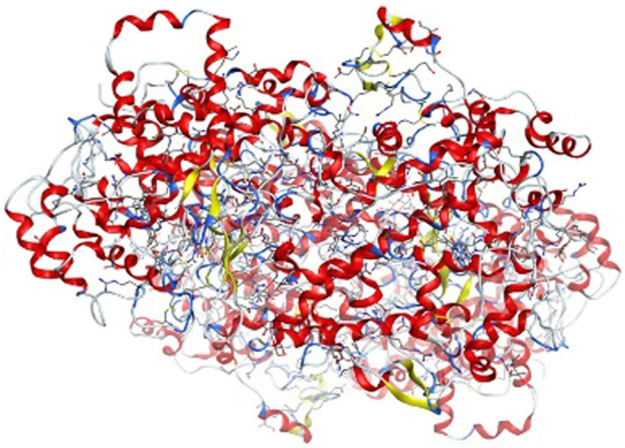

FIGURE 3 3D 1PXX structure

generated; it will actively bind to the amino acids and form a more stable complex. In an attempt to understand the molecular basis behind the anti-inflammatory activity of *M. indica* fruits peel extract and the observed COX-2 inhibitory activity, the eighteen identified metabolites in the extract as in Figure S2 were docked in the active site of COX-2 obtained from the Protein Data Bank, PDB ID: 1PXX, using MOE software version 2014.0901 (Figure 3). The crystal structure of a complex of diclofenac, ibuprofen, and aspirin with murine COX-2 was determined. Figure 4 demonstrates that diclofenac binds to COX-2 in an inverted conformation with its carboxylate group hydrogen-bonded to Tyr-385 and Ser-530.[33] Ibuprofen was bonded to Tyr-385 while aspirin was bonded to Ser-530 with energy consumptions of −6.4806 and −5.2969, respectively, thus diclofenac was found to possess the best binding activity to COX-2 (Figure 5).

As shown in Table 4, most of the metabolites were active ligands and bound to the amino acids of COX-2 with low energy consumptions. Quercetin and its glycosides bound to Ser 530 amino acids but not to Tyr 385 where the least energy consumption of them was that of quercetin-3-*O*-glucoside (−6.1772), while mangiferin actually bound to both Tyr 385 and Ser 530 with energy consumption of −6.1825. Digalloyl glucose showed the least energy consumption (−7.7192) but it is also bound to Ser 530 only with no interactions with Tyr 385. It is clear that quercetin-3-*O*-glucoside, mangiferin, and digalloyl glucose showed good binding interactions at the protein active sites and formed stable complexes as depicted in Figure 6. Hence, believed to engage in inhibitory activity against the COX-2 enzyme.

To our knowledge, this is the first report to evaluate the beneficial effects of *M. indica*, *C. sinensis*, *C. melo*, and *P. granatum* fruits peel extracts in the treatment of OA in rat model. Our results showed that the different fruit peels extracts exhibited a chondroprotective effect in the rat OA model via downregulation of mRNA expressions for COL1A1, anti-inflammatory, and anti-oxidant effects, thus could be potentially used for treatment of OA which is in the same line of our hypothesis.

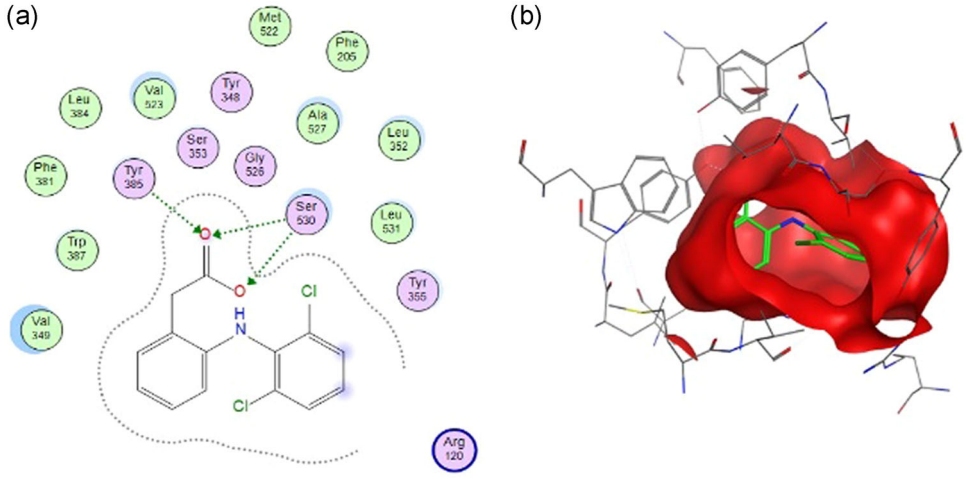

FIGURE 4 Interactions of diclofenac with 1PXX (a: 2D and b: 3D)

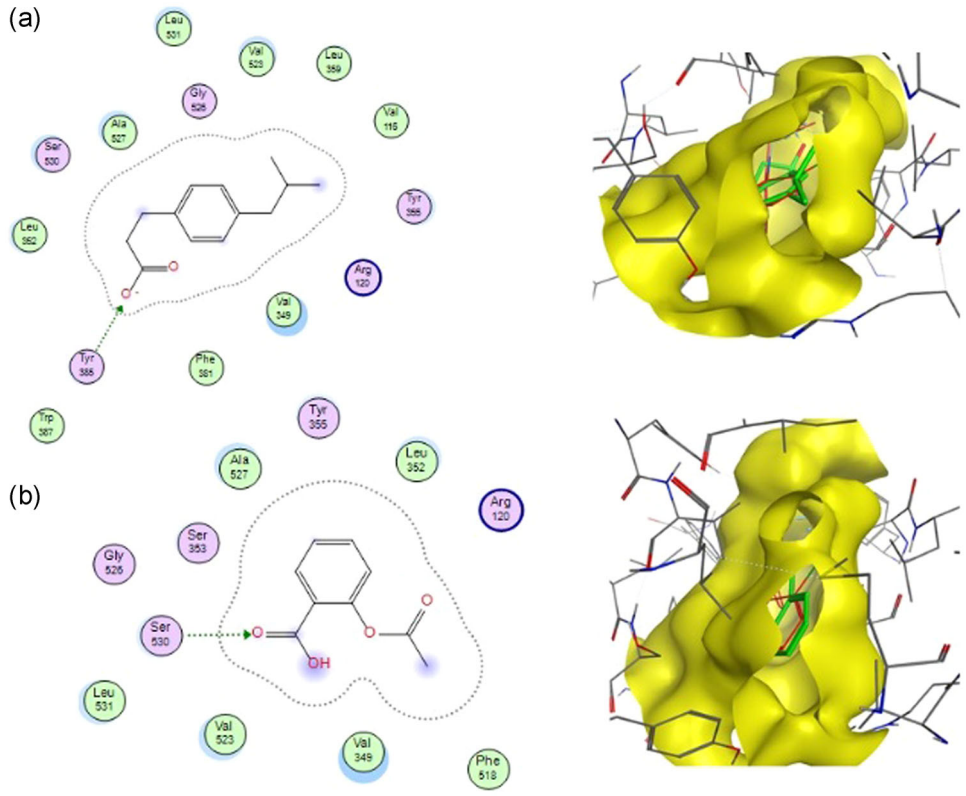

FIGURE 5 Interactions of ibuprofen (a) and aspirin (b) with 1PXX (2D and 3D)

To elaborate the mechanism of action of *M. indica*, *C. sinensis*, *C. melo*, and *P. granatum* fruits peels extracts; we assessed the levels of IL-6, COX-2, PPARγ, α-SMA, oxidative biomarkers as well as the genetic expression of COL1A1 in our OA model.

Our results demonstrated that the fruit peel extracts dose-dependently inhibit inflammation in the OA rat model as measured by decreasing the levels of IL-6, COX-2, and NO and elevating the level of PPARγ, also downregulate the expression of COL1A1, as well as reduce the fibrosis and differentiation by measuring the level of α-SMA which was significantly decreased, also, decrease the oxidative stress in OA articular cartilage that was measured by decreasing level of MDA and increasing level of GSH.

The mean levels of IL-6, COX-2, and NO were significantly increased in the OA group compared to the control group, p-value was < 0.001, the treatment with different fruit peels extracts at the two doses reduced the levels of IL-6, Cox-2, and NO compared with OA group, p-value was < 0.01. These results could explain partly the effect of the four fruit peels extracts observed in our study.

TABLE 4 Docking energy scores (S) in kcal/mol and the interactions of the COX-2 receptor proteins with diclofenac and metabolites identified in *Mangifera indica* fruits peels extract

Compound	Docking score (kcal/mol)	Hydrogen bonding
Diclofenac	−6.0104	Tyr 385, Ser 530, Ser 530
Ibuprofen	−6.4806	Tyr 385
Aspirin	−5.2969	Ser 530
Quercetin	−4.2115	Ser 530, Ser 530
Quercetin-7-*O*-glucoside	−2.3207	Ser 530, Ala 527, Ser 353, Arg 513, Gln 192, Phe 518
Querecetin-3-*O*-glucoside	**−6.1772**	**Ser 530, Ser 530, Met 522, Ala 527, His 90**
Quercetin-3-*O*-rhamnoside	−4.9279	Ser 530, Ser 530, His 90
Quercetin-3-*O*-glucosyl glucoside	4.1620	Ser 530, Arg 120, Arg 120, Arg 120, Tyr 355, Gln 192, His 90, Met 522
Peonidin-3-*O*-glucoside	−5.7220	Ser 530, Met 522, Ala 527
Peonidin-3-*O*-malonyl glucoside	−3.8574	Ser 530
Malvidin-3-*O*-acetyl glucoside	−4.9651	Tyr 385, Ser 353, Arg 120, Arg 120, Leu 352
Procyanidin A	−0.6380	No interactions
Mangiferin	**−6.1825**	**Tyr 385, Ser 530, Gly 526**
Mangiferin gallate	−1.5357	Ser 530, Ser 530, Gly 526
Maclurin-3-*C*-glucoside	−4.8836	Ser 530, Arg 120
Valoneic acid dilactone	−5.6354	Ser 530, Ser 530
Gallic acid	−4.8380	Ser 530, Arg 120, Arg 120
Theogallin	−5.7855	Ser 530, Ser 530, Val 523
Ethyl gallate	−5.9856	Ser 530
Ethyl *p*-trigallate	−2.9252	Ser 530, Ser 530, Gln 192
Digalloyl glucose	**−7.7192**	**Ser 530, Ser 530, Gly 526**

Note: The bold values showed the best binding interactions and formed stable complexes. Hence, believed to engage in inhibitory activity against the COX-2 enzyme.

In the articular cells, IL-1β and TNF amplify OA process by stimulating the production of pro-inflammatory cytokines such as IL-6 which in turn upregulates the expression of genes encoding inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and microsomal prostaglandin E synthase 1 which stimulates the production NO and prostaglandin E2 (PGE2), so contributing to the articular inflammation and degradation.[34]

Our results are in agreement with the previous studies performed by Pan et al. which found elevated serum IL-6 production in the AIA group. In addition, intervention from acamprosate at different doses ameliorated the increased levels IL-6 in serum.[35]

Our study showed that the level of PPARγ in cartilage tissue in the OA group was decreased compared to the control group, p-value was < 0.001, on the other hand, its level was significantly raised in *M. indica* 400, *C. sinensis* 400, *C. melo* 400, *P. granatum* 400 and Voltaren groups compared to OA group (p-value < 0.01)

PPARγ plays an important role in the pathogenesis of OA, RA, and other chronic inflammatory diseases. The expression level of PPARγ was reduced in OA cartilage when compared to control one, thus these findings confirm that the downregulation of PPARγ will result in increased expression of inflammatory cytokines.

Qu et al. found that mangiferin suppressed IL-1β-induced inflammatory mediator production. These results suggested that mangiferin exhibited anti-inflammatory effects in IL-1β-stimulated chondrocytes by inhibiting inflammatory mediators release such as PGE2 and NO where these results were involved to the activation of PPARγ.[36]

To further understand the molecular events underlying cartilage destruction after MIA injection, the expression of COL1A1 was measured in which the *M. indica*, *C. sinensis*, *C. melo*, and *P. granatum* treatments dose-dependently downregulated the rats' cartilage mRNA expressions for COL1A1. In addition to the high dose of *M. indica* (400 mg/kg) showed a prominent effect over all other peels extracts.

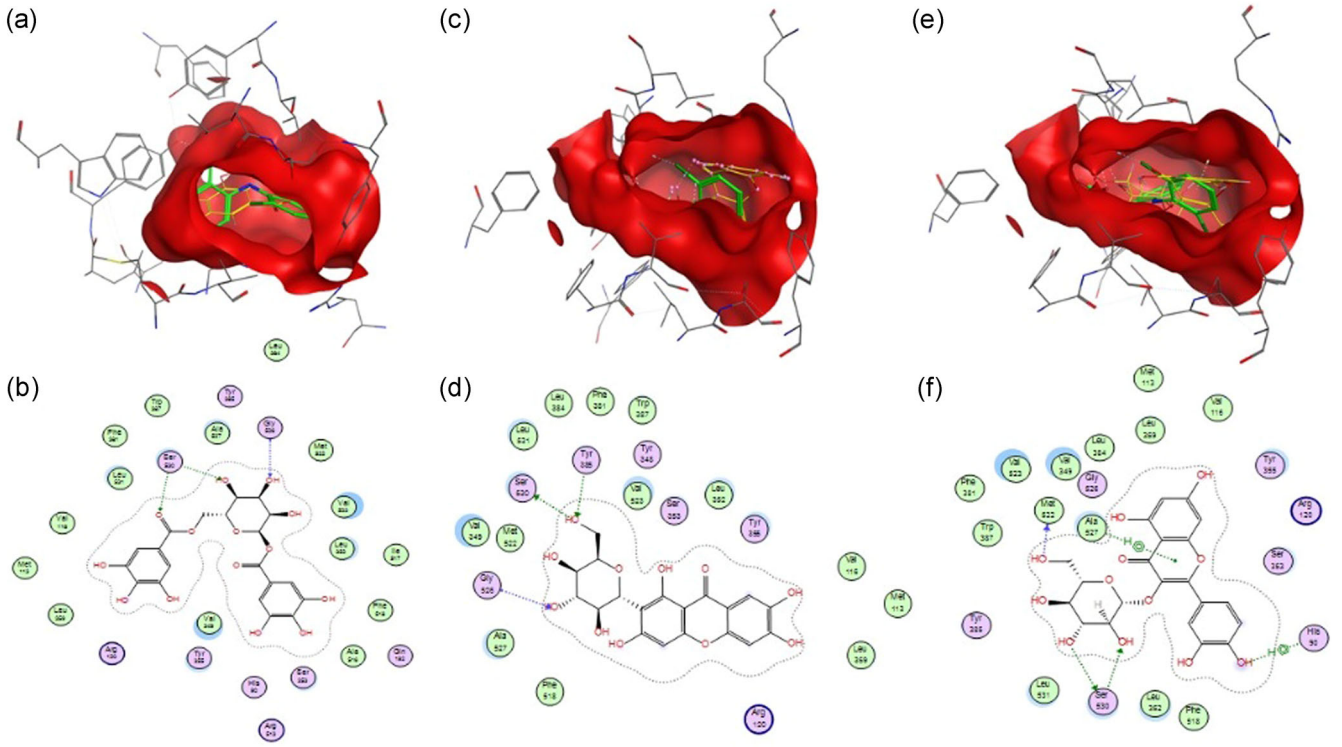

FIGURE 6 Interactions of querecetin-3-*O*-glucoside, mangiferin, and digalloyl glucose with 1PXX. (a) 2D of quercetin-3-*O*-glucoside, (b) 3D of quercetin-3-*O*-glucoside, (c) 2D of mangiferin, (d): 3D of mangiferin, (e): 2D of digalloyl glucose, (f): 3D of digalloyl glucose

COL1A1 is a fibroblastic marker that is expressed in the dedifferentiated chondrocytes. A significant increase in COL1A1 was observed in higher OA grades, meanwhile, the ratio of COL2A1/COL1A1 decreased significantly with OA severity (grade 0–5) as in the study carried out by Zhong et al.[37] Another study performed by Gosslau et al. showed the anti-inflammatory activity of orange peels extracts at different doses on carrageenan-induced mouse hind paw edema model by downregulation of expression of inflammatory genes as COXII, TNF-α, ICAM-1, NFκB, IL-1β, IL-6, and IL-8 which was related to its content polymethoxylated flavones (PMFs).[38]

Pomegranate extract showed anti-inflammatory action through inhibiting the COX-2 enzyme and so the production of PGE2.[39]

In this study; the mean level of α-SMA was significantly increased in the OA group compared to the control group $p < 0.001$, while a significant reduction was observed in *M. indica* 200 and 400, *C. sinensis* 400, *C. melo* 200 and 400, *P. granatum* 200 and 400, and Voltaren 30 groups when compared to OA group at $p < 0.01$.

α-SMA is formed from the fibroblast during the healing process when the latter acquires a contractile phenotype, myofibroblast cells are involved in morphogenesis, inflammation, wound healing, fibrosis, and oncogenesis in many tissues and organs through ECM reorganization by stimulating the production of inflammatory mediators. After the repair process is completed; these cells disappeared by apoptosis while their presence stimulates impaired repair mechanisms which leads to excessive contraction, ECM secretion, and finally fibrosis.[40]

In line with our results, a study carried out by Wang et al. who performed a study on female rats after injection with different doses of MIA into the upper compartment where α-SMA, markers of proliferation and fibrosis was upregulated in the MIA group.[41]

Our results showed that the mean level of MDA was significantly increased in the OA group compared to the control group at $p < 0.001$, while it was reduced in *M. indica* 200 and 400, *C. sinensis* 200 and 400, *C. melo* 200 and 400, *P. granatum* 200 and 400, and Voltaren 30 groups when compared to OA group at $p < 0.01$.

Lipid peroxidation leads to production of toxic compounds such as MDA and so decreasing levels of anti-oxidants. these results come in agreement with Gondi and Rao[42] as they also proved that *M. indica* fruits peels extracts decreased the MDA levels in diabetic rats.

The antioxidants are considered a set of compounds that may be present in different nutraceuticals, which act in synchrony with the human body, consuming free radicals and maintaining the body's health.[30,43] To evaluate the antioxidant effect, we measured GSH level, our results showed that GSH level was significantly reduced in OA group compared to control group, $p < 0.001$, also *M. indica* 400 and Voltaren 30 groups significantly restored the decreased GSH levels compared to OA group at $p < 0.01$. These results come in agreement with Wei et al. who proved that *P. granatum* fruits peels extracts increased the GSH levels.[44] It is well established that antioxidants promote the anti-inflammatory mechanisms in which the free radicals were involved.[45] Thus, these fruit peels extracts are suggested to possess chondroprotective activities through downregulation of COL1A1 and anti-inflammatory mechanisms.

3 | CONCLUSION

This study well-defined the concept of employing different fruit peels as novel alternative medicinal agents in the treatment of osteoarthritis, with emphasis on *M. indica*, *C. sinensis*, *C. melo*, and *P. granatum*. The best chondroprotective effect in the OA rat model was exhibited by *M. indica* fruits peels extract. This effect is believed to be mediated via the downregulation of COL1A1 in articular cartilage, anti-oxidant, and anti-inflammation. It is intriguing to speculate that the major metabolites of *M. indica* fruits peel extracts, especially, quercetin-3-O-glucoside, mangiferin, and digalloyl glucose are actually engaged in the inhibitory activity against the COX-2 enzyme.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | Plant material collection and extraction procedures

The fruits of *M. indica* L. (cultivar Zebda) family Ancardiaceae, *C. sinensis* L. family Rutaceae, *C. melo* L. (cultivar Cantalupo) family Cucurbitaceae, and *P. granatum* L. family Punicaceae were purchased from the Faculty of Agriculture farms, Cairo University during 2021. The taxonomical identity was kindly verified by the vegetables and fruits department, Faculty of Agriculture, Cairo University. Their peels were isolated and any flesh present there was totally removed, then dried under shade, in a clean dust-free environment. The peels were ground and macerated in 70% ethanol till exhaustion. The hydro-ethanolic extracts were concentrated under reduced pressure at a temperature not exceeding 50°C and placed in a desiccator in tightly closed amber glass containers for chemical and biological investigations.

4.1.2 | UPLC-ESI/MS apparatus

All samples were prepared at a concentration of 40 µg in 1 ml HPLC grade methanol, filtered using a membrane disc filter (0.2 µm), and degassed by sonication before injection. UPLC–ESI/MS analyses were carried out on an Agilent® 1100 Series using ACQUITY UPLC - BEH C18 column (1.7 µm−2.1 × 50 mm, i.d.), with an integrated pre-column. For standard analyses, samples injection volumes (10 µl) were eluted using gradient mobile phase comprising two eluents: eluent A is nano-pure H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid with the flow rate of 0.2 ml/min in 35 min. A XEVO TQD triple quadruple instrument, Waters® Corporation, Milford, MA01757, USA mass spectrometer connected to a PDA detector with standard flow cell (10 mm path length, 14 µl volume, 40 bar maximum pressure) was used for mass spectrometric analysis. ESI interface was used in both negative and positive ion modes under the following conditions: drying and nebulizing gas, N_2; capillary temperature, 250°C; spray voltage, 4.48 kV; capillary voltage, 39.6 V; tube lens voltage, 10.00 V; and full scan mode in mass range *m/z* 100–2000. The peaks and spectra were processed using the MassLynx 4.1® software and tentatively assigned by comparing their retention times (Rt) and mass spectrums with reported data.

4.2 | Pharmacological/biological assays

4.2.1 | Experimental animals

Sixty-six male albino rats weighing 180 ± 20 g were used in this study. Before experimentation, these rats were kept in an air-conditioned animal room at 22°C with a 12 h light/dark cycle (four rats per cage) at the animal house of MSA University. Commercial pellet and tap water ad libitum were given to rats for 2 weeks for acclimation before being used.

Animal care and handling was performed in conformance with approved protocols of MSA University and Egyptian Community guidelines for animal care. The Research Ethics Committee at MSA Faculty of Pharmacy approved the protocol of this study. Also complied with the ARRIVE guidelines and were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

4.2.2 | Induction of osteoarthritis using MIA

Rats were lightly anesthetized (3% isoflurane in O_2 at 1.5 l/min) and then a single intra-articular (i.a.) injection of 2 mg of MIA (Sigma-Aldrich) was performed through the infra-patellar ligament into the joint space of the right knee of rats in a total volume of 50 µl saline 5.

4.2.3 | Experimental design and animal allocation

Male albino rats were randomly allocated into 11 groups of six animals each (Table 5).

4.2.4 | Drugs, chemicals, and solvents

MIA was ordered from Sigma-Aldrich, Diclofenac Sodium (Voltaren®) was purchased from Novartis and 95% ethanol of analytical grade were purchased from Al-Gomohorya Pharmaceuticals.

4.2.5 | Blood samples and biochemical analysis

At the end of the study, rats were fasted overnight, anesthetized with thiopental sodium (50 mg/kg) and blood samples were collected in

TABLE 5 Animal grouping and allocation

Group 1	Normal control group injected (i.a) by 50 µl saline in the right knee.
Group 2 (OA)	Rats injected (i.a) of 2 mg of MIA in a total volume of 50 µl saline in the right knee.
Group 3 (*Mangifera indica* 200)	Osteoarthritic rats treated with extract of *M. indica* 200 mg/kg/day, orally for 28 days.
Group 4 (*M. indica* 400)	Osteoarthritic rats treated with extract of *M. indica* 400 mg/kg/day, orally for 28 days.
Group 5 (*Citrus sinensis* 200)	Osteoarthritic rats treated with extract of *C. sinensis* 200 mg/kg/day, orally for 28 days.
Group 6 (*C. sinensis* 400)	Osteoarthritic rats treated with extract of *C. sinensis* 400 mg/kg/day, orally for 28 days.
Group 7 (*Cucumis melo* 200)	Osteoarthritic rats treated with extract of *C. melo* 200 mg/kg/day, orally for 28 days.
Group 8 (*C. melo* 400)	Osteoarthritic rats treated with extract of *C. melo* 400 mg/kg/day, orally for 28 days.
Group 9 (*Punica granatum* 200)	Osteoarthritic rats treated with extract of *P. granatum* 200 mg/kg/day, orally for 28 days.
Group 10 (*P. granatum* 400)	Osteoarthritic rats treated with extract of *P. granatum* 400 mg/kg/day, orally for 28 days.
Group 11 (Voltaren 30)	Osteoarthritic rats treated with Voltaren 30 mg/kg/day, orally for 28 days.

Note: Doses were selected based on previously published results of toxicity studies in similar species.[46]

the morning (5 ml per rat). Blood samples were centrifuged at 3000 rpm for 15 min after 30 min of collection and stored at −80°C until analyzed. Serum interleukin-6 (IL-6) was measured using the rat enzyme immunoassay kits (Immuno-Biological Laboratories, Inc.), Catalog no. BE71953, biotin conjugated anti-rat IL-6 antibody dilution was at 1:100 according to manufactured instructions.

4.2.6 | Tissue preparation and biochemical analysis

Animals were euthanized by cervical dislocation, and then articular cartilage tissue was rapidly removed from each rat and stored in liquid nitrogen, part was rinsed in ice-cold PBS (pH 7.0–7.2), and then ground using a tissue homogenizer (Biospec Product, mini-BeadBeater-8), the tissue fragments were dispersed in 650–800 µl of lysis buffer containing 100 µg of BSA fraction V, 100 µg Zwittergent-12, and 50 µg of gentamicin/ml, 10 mM HEPES buffer, 1 µg aprotinin and leupeptin/ml, 0.1 µm EDTA, then the incubation mixture was placed on ice and was sonicated for 20–30 s and centrifuged at 4000 rpm for 15 min. The supernatant was collected for measuring cyclooxygenase-2 level (COX-2) (CUSABIO), Catalog no. CSB-E13516r, biotin antibody dilution was at 1:100 PPARγ (MyBioSource, Inc.), Catalog no. MBS 761842, biotin detection antibody dilution was at 1:100, α-SMA (CUSABIO), Catalog no. CSB-E14322r, biotin antibody dilution was at 1:100 using the rat enzyme immunoassay kits and MDA, NO, and GSH (BioDiagnostic, diagnostic and research reagents, Egypt) using the colorimetric method according to manufacturer's instructions.

4.2.7 | Gene expression analysis by RT-qPCR

Collagen 1 alpha 1 RNA from the articular cartilage tissue of the rats was extracted with RNeasy Mini Kit (Qiagen, GmbH), RNA extracted from tissue homogenates was eluted in 45 µl of nuclease-free distilled water. The RNA of each sample was reverse-transcribed with RT2 First Strand kit (Qiagen). Quantification of collagen 1 alpha 1 PCR was carried out by using of collagen 1 PCR fluorescence quantitative diagnostic kit (Brilliant SYBR Green Master Mix). The primers were purchased from the Qiagen PCR array and possess the proprietary information of the primer sequences. Thermal cycling and fluorescence detection were performed using a Rotor-Gene Q5 plex real-time Rotary analyzer (Corbettlife Science).

4.2.8 | Histopathological examination

At the end of the study, rats were fasted overnight, anesthetized with thiopental sodium (50 mg/kg), and knee joints were dissected, rinsed in saline. Specimens were fixed in 10% formalin and then joints were decalcified in nitric oxide for 4 days, routinely processed, and embedded in paraffin. Five microns sections were cut and stained with hematoxylin and eosin (H&E).

4.2.9 | Statistical analyses

All data were expressed as mean ± SD and analyzed using Prism program version 6. For all parameters, comparisons among groups ($N = 6$) were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. All p-values reported are two-tailed and $p < 0.05$ was set as the level of significance.

4.2.10 | Ethics approval

Animal care and handling were performed in conformance with approved protocols of MSA University and Egyptian Community

guidelines for animal care. The ethical committee approval number for this manuscript was: BP1/Ec1/2021PD.

4.3 | Molecular docking

4.3.1 | In silico studies of the identified *M. indica* metabolites

The molecular docking was performed using Molecular Operating Environment MOE software version 2014.0901. The 18 identified metabolites in *M. indica* peels extract were docked in the active site of COX-2 obtained from the Protein Data Bank as a 3D cocrystal structure with a native ligand (PDB ID: 1PXX) (Figure 3). Before performing the docking, protein–ligand complexes obtained from the Protein Data Bank were prepared as follows: three chains from the enzyme were eliminated, 3D protonation; partial charges were calculated using Amber 99 force field; hydrogens were hidden; crystallization water was deleted; the active sites were isolated; the amino acids involved were identified and ligand interactions with the active site amino acids were studied. Validation was performed by re-docking of the native ligand and RMSD value of Å (1.0381) was procured. The 3D structures of 18 metabolites (used as experimental ligands) were built using MOE builder and structures were optimized under the same conditions of enzyme optimization; then, subjected to conformation analysis using systematic search, and the least energetic conformers were selected and used for molecular docking. Docking was performed using the Alpha triangle placement method and default parameters were not changed. Poses were prioritized based on the affinity London dG scoring method and those with the best affinity were used for the study of ligand-receptor interactions.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Mahitab H. El Bishbishy http://orcid.org/0000-0002-0755-7229

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