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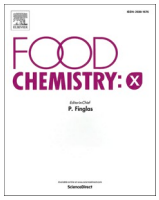

Unlocking the therapeutic treasure of pomegranate leaf: A comprehensive review on phytochemical compounds, health benefits, and future prospects

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ABSTRACT

The exploration of sustainable and valuable by-products from industrial and agricultural processes is increasingly recognized for its economic, environmental and health advantages. This review examines the phytochemical constituents, biological properties, current applications and future directions of pomegranate (*Punica granatum* L.) leaf (PGL). PGL exhibits broad biological activities, aiding in managing health conditions like chronic diseases, cancer, diabetes, obesity, and neurological disorders. Anti-cancer and anti-diabetic effects are demonstrated *in vitro* and *in vivo* using animal models. Anti-inflammatory and neuroprotective properties are also observed in cell cultures and animal studies. Its anti-microbial properties show efficacy against pathogens. However, variability in phytochemical composition due to different extraction methods and environmental conditions poses challenges for standardization. The review underscores the urgent need for comprehensive human clinical trials to confirm PGL's therapeutic benefits and safety, calling for future research to fully harness PGL's potential as a sustainable and bioactive compound in various industrial applications.

1. Introduction

The *Punica granatum L.*, commonly known as the pomegranate, is a botanical *gem* with origins in the ancient India, dating back to the fourth century. Its adaptability and resilience have enabled it to thrive across various agro-climatic regions (Ge et al., 2021; B. Singh, Singh, Kaur, & Singh, 2018), with primary cultivation in India, Iran, China, the USA, and Turkey (Ranjha et al., 2021). Pomegranates belong to the Punicaceae family and exhibit extensive genetic diversity, resulting in variations in their phytochemical composition and bioactive properties. The Wonderful variety is the most studied for its significant bioactive compounds, while other notable varieties like Gabsi, Mollar de Elche, Ganesh, Nana and Ruby have also demonstrated substantial bioactive potential, particularly in *in vitro* studies (Melgarejo-Sánchez et al., 2021).

Every part of the pomegranate tree, from its aril, peel, and seed to its flower, leaf, bark, and root, serves as a testament to nature's bounty, brimming with therapeutic properties (Melgarejo-Sánchez et al., 2021; Ranjha et al., 2021). However, the global research community has predominantly focused on the fruit, namely its juice, due to its rich bioactive components and plenty of health benefits, even the peel and seeds are explored gradually (Baghdadi, Shidfar, & Dehnad, 2023; B. Singh et al., 2018).

There are two main classifications of the pomegranate tree: ornamental and edible. The edible variety is particularly noteworthy for its vibrant red flowers, succulent fruits filled with red juice, soft seeds, and most importantly, its medicinal potential (Ge et al., 2021). It's essential to recognize that the health advantages are not confined to the fruit alone; the non-edible parts also hold significant potential, broadening the horizons for innovation and application (Amri et al., 2017).

In the face of pressing challenges that modern food systems face, such as significant greenhouse gas emissions, biodiversity loss, and

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health concerns, there's a growing shift towards sustainable and health-centric alternatives (Willett, Rockstrom, & Loken, 2020). The non-edible components of the pomegranate, especially when viewed through the lens of a biorefinery approach, are gaining traction. They not only offer valuable health byproducts but also promote a holistic use of biomass, aligning with environmental and bio-economic goals (Kiraz, Neergheen-Bhujun, Rummun, & Baran, 2016; Sanna et al., 2021).

Recent studies (Celiksoy & Heard, 2021; Haji, Shahmoradi Ghaheh, & Indrie, 2023) have highlighted the substantial waste generated from pomegranate cultivation, with leaves constituting a significant portion. These leaves, often discarded as by-products of pruning, hold immense untapped potential, being rich in bioactive compounds suitable for pharmaceutical, nutraceutical, and cosmetic industries. It has been estimated that for every ton of harvested pomegranates, approximately 150–200 kg of leaves are discarded (Haji et al., 2023). Given that global pomegranate production exceeded 3 million tons in 2020 (FAO, 2021), this translates to an estimated 450,000 to 600,000 tons of pomegranate leaf waste annually. Thus, investigating pomegranate leaf (PGL) as a renewable agro-industrial waste becomes increasingly important for reducing environmental impact and providing economic benefits by converting waste into valuable products.

Historical records from esteemed international pharmacopoeias and traditional medicine systems, like the Ayurveda and Traditional Chinese Medicine, highlight the diverse uses of pomegranate leaves. From treating fungal infections in Bangladesh to aiding digestion in Morocco, these leaves have found varied applications across cultures (Table S1). Recent studies have further substantiated these traditional uses, shedding light on the medicinal properties of pomegranate leaves, including their antioxidant, anti-inflammatory, and bioactive compound profiles (Pottathil et al., 2020; M. Yu et al., 2023).

Enriched with a plethora of phenolic components, proteins, steroids, and other vital minerals (Bhinge et al., 2021; Farag, Abdel-Latif, Emam, & Tawfeek, 2014; Snehal Nitin Mestry, Dhodi, Kumbhar, & Juvekar, 2017; Pottathil et al., 2020), pomegranate leaves offer a myriad of health benefits (Table S2), including protection against diabetes, obesity, infections, and even chemotherapy-induced side effects (Acquadro et al., 2020; Deng et al., 2018; Pottathil et al., 2020). While some commercial products in the form of powdered capsules, tablets, or beverages, have started incorporating these leaves in the USA an China, a significant portion remains underutilized (Al-Muammar & Khan, 2012; Zarfeshany, Asgary, & Javanmard, 2014).

This review aims to address the untapped potential of pomegranate leaves, exploring their bioactive ingredients, antioxidant properties, health benefits, and toxicological effects. By synthesizing research from the past two decades, we hope to pave the way for future dietary supplements, medicinal innovations, and clinical applications, promoting a more sustainable and health-conscious future.

2. Phytochemicals of pomegranate leaves

Pomegranate leaf (PGL) is recognized as a rich source of various bioactive compounds, as detailed in Table 1. These compounds encompass phenolic substances, predominantly phenolic acids, flavonoids, and tannins. The presence and concentration of these components in PGL are influenced by factors such as the specific cultivar, geographical origin, extraction techniques, and seasonal variations, as highlighted in Table S3. Notably, phenolic acids like gallic acid and ellagic acid are frequently identified in PGL (Akkawi, Abu-Lafi, & Abu-Remeleh, 2019; Pottathil et al., 2020). Key flavonoids present in PGL include apigenin, luteolin, quercetin, rutin, catechin, and kaempferol, along with their derivatives (Ankita, Deepti, & Nilam, 2015; Farag et al., 2014; Snehal Nitin Mestry et al., 2017; Pottathil et al., 2020). Furthermore, PGL is rich in both hydrolysable tannins (HTs) and condensed tannins (CTs) (Xiang et al., 2008). Fig. 1 provides a visual representation of the chemical structures of the primary compounds found in PGL.

2.1. Phenolic acids

Phenolic content in Pomegranate Leaf Extracts (PGLE) varies due to factors like cultivars, regional differences, and seasonal changes. The Kesar and Ganesh cultivars from Karnataka, India, exhibited distinct total phenolic content (TPC) levels in their aqueous (AQ) and acetone (AC) PGLE, respectively (Kolar, Lingasur, Kumathalli, & Gurikar, 2021). In Tunisia, the Gabsi cultivar's PGLE had a higher TPC (118.9 mg GAE/100 g) than the Nabli cultivar (79.7 mg GAE/100 g) (Fellah, Bannour, Rocchetti, Lucini, & Ferchichi, 2018). Additionally, the Nana variety from Tunisia showed regional variations in TPC, with Mahdia's PGLE (250 mg GAE/g) being lower than Moknine's (310 mg GAE/g) (Amri et al., 2017). Zhang, Gao, Zhang, Liu, and Yu (2010) noted a decline in TPC during the early growth stages of PGL, followed by a gradual increase until September's end.

Extraction methods and solvents play a pivotal role in determining PGLE's phenolic acid content. For instance, successive extraction proved more effective in extracting antioxidants from PGL than individual methods like cold percolation or decoction (Kaneria, Bapodara, & Chanda, 2012). A study from Turkey highlighted that aqueous PGLE had higher concentrations of gallic acid (6.92 mg/g) and chlorogenic acid (22.25 mg/g) than methanol extracts (0.73 mg/g and 3.32 mg/g, respectively) (Uysal, Zengin, Aktumsek, & Karatas, 2016). Additionally, certain phenolic acids were exclusive to specific solvents: *p*-hydroxybenzolic acid and benzoic acid were only in methanolic PGLE, while ferulic acid was exclusive to water extracts (Uysal et al., 2016).

Interestingly, some research indicates that PGL have a superior TPC compared to other medicinal or edible plants and even other pomegranate parts. In Turkey, methanolic PGLE had the highest TPC among tested fruit tree leaves (Uysal et al., 2016). Another study showed that Thai ethanolic PGLE had a higher TPC than nine other edible plants (Kaewnarin, Niamsup, Shank, & Rakariyatham, 2014). Furthermore, the Gabsi cultivar's methanolic PGLE from Tunisia had a notably high phenolic acid concentration (721.9 mg FAE/kg), contrasting sharply with the concentrations in peels and flowers (164.5 and 173.3 mg FAE/kg, respectively) (Fellah et al., 2018; Fellah et al., 2020).

2.2. Flavonoids

Flavonoid concentrations in pomegranate leaf (PGL) differ depending on the cultivar, origin, and seasonal changes. The ethanolic extracts from Indian PGL showed a concentration of 7.69 mg RE/g in the Ganesh cultivar, while the Kesar cultivar had 3.58 mg RE/g (Kolar et al., 2021). Rutin, a notable antioxidant, was found in varying amounts in PGL sourced from Tunisia, Tiruchirappalli and Karnataka in India, and Turkey, with concentrations of 3.56, 1.12, 0.29, and 0.05 mg/g, respectively (Durgadevi, Marueen, Gowri, & Ramamurthy, 2018; Ramamurthy & Marueen, 2018; Trabelsi et al., 2020). Brazilian PGL contained flavan-3-ols (like catechin, epicatechin, and epigallocatechin 3-gallate), flavonols (such as quercetin and kaempferol), and flavonoid glycosides (including kaempferol-3-O-glycoside and kaempferol-arabinoside) (Marques et al., 2016; Pinheiro et al., 2018). In contrast, Indian PGL had quercitrin and cosmosiin (S. Mestry et al., 2020). The total flavonoid content (TFC) of methanolic PGL from Maharashtra, India (collected in January 2014) was 126.667 mg RE/g. Meanwhile, ethanolic PGL from Gujarat, India (collected in August 2012) had a TFC of 102.02 mg QE/g (Ankita et al., 2015; S. N. Mestry & Juvekar, 2017).

The extraction method and solvents used significantly influence flavonoid content. For instance, rutin was only detected in aqueous PGLE, while apigenin was exclusive to methanolic PGLE (Uysal et al., 2016). The Gabsi cultivar, when extracted using TOF, had a TFC of 492.88 mg QE/g, which was higher than other solvents like hexane, chloroform, ethyl acetate, ethanol, or water (Trabelsi et al., 2020). Hydroalcoholic PGLE had a notably higher TFC than aqueous extracts, but the latter had more flavonols than the former (Mohammed, Mohamed, Ahmed, & Mariod, 2020). When using solvents like hexane,

Table 1
Formula and molecular weight of individual phytochemical compound reportedly present in pomegranate leaves.

ID	Category	Compound Name	Formula	Molecular Weight	Reference
1	Ellagitannins and gallotannins	Castalagin	$C_{41}H_{26}O_{26}$	934.63	Pinheiro et al. (2018)
2		Corilagin	$C_{27}H_{22}O_{18}$	634.45	Akkawi et al. (2019); Angamuthu et al. (2019)
3		Gallagic acid	$C_{28}H_{14}O_{18}$	638.39	Orgil et al. (2014)
4		Granatin A	$C_{34}H_{24}O_{23}$	800.54	Angamuthu et al. (2019); Toda et al. (2020)
5		Granatin B	$C_{34}H_{28}O_{27}$	952.64	Akkawi et al. (2019); Swilam and Nematallah (2020); Toda et al. (2020)
6		5-*O*-galloyl-punicacortein D	$C_{54}H_{34}O_{34}$	1222.8	El-Toumy and Rauwald (2002)
7		Pentagalloyl glucose	$C_{41}H_{32}O_{26}$	940.70	Lakshminarayanashastry et al. (2019)
8		Punicafolin	$C_{41}H_{30}O_{26}$	938.66	Tanaka et al. (1985)
9		Punicalagin	$C_{48}H_{28}O_{30}$	1084.7	Li et al. (2016); Orgil et al. (2014)
10		Punicalin	$C_{34}H_{22}O_{22}$	782.53	Pinheiro et al. (2018)
11		Strictinin	$C_{27}H_{22}O_{18}$	634.45	Angamuthu et al. (2019); Tanaka et al. (1985)
12		Tannic acid	$C_{76}H_{52}O_{46}$	1701.2	Yu et al. (2017)
13		Tellimagrandin I	$C_{34}H_{26}O_{22}$	786.56	Hussein et al. (1997)
14		Tercatain	$C_{34}H_{26}O_{22}$	786.56	Hussein et al. (1997)
15	Ellagic acid and derivatives	Ellagic acid	$C_{14}H_6O_8$	302.19	Elfiky (2018); Li et al. (2016); Pinheiro et al. (2018)
16		Ellagic acid, 3,3'-di-*O*-methyl	$C_{16}H_{10}O_8$	330.25	Marques et al. (2016)
17		Ellagic acid, 3,4,3'-tri-*O*-methyl	$C_{17}H_{12}O_8$	344.27	Akkawi et al. (2019)
18		Eschweilenol C	$C_{20}H_{16}O_{12}$	448.33	Akkawi et al. (2019)
19	Flavonoids and derivatives	Apigenin	$C_{15}H_{10}O_5$	270.05	Pottathil et al. (2020); Angamuthu et al. (2019); Elfiky (2018); Uysal et al. (2016)
20		Apigenin-4′-*O*-β-D-glucoside	$C_{21}H_{20}O_{11}$	448.32	Nostro et al. (2016); M. A. M. Nawwar et al. (1994)
21		Apigenin-7-*O*-β-D-glucoside (Cosmosiin)	$C_{21}H_{20}O_{10}$	432.40	Elfiky (2018); Acquadro et al. (2020); Mestry et al. (2020)
22		Catechin	$C_{15}H_{14}O_6$	290.27	Farag et al. (2014)
23		Cyanidin-3,5-diglucoside	$C_{27}H_{31}O_{16}$	611.52	Akkawi et al. (2019)
24		5,7-Dihydroxyflavone (Chrysin)	$C_{15}H_{10}O_4$	254.24	Elfiky (2018)
25		Eriodictyol-7-*O*-α-L-arabinofuransyl (1-6)-β-D-glucoside	$C_{26}H_{30}O_{15}$	582.51	Chauhan and Chauhan (2001)
26		Gossypetin 8-glucoside (Gossypin)	$C_{21}H_{20}O_{13}$	480.38	Lakshminarayanashastry et al. (2019)
27		Kaempferol	$C_{15}H_{10}O_6$	286.24	Elfiky (2018); Marques et al. (2016)
28		Kaempferol 3-*O*-β-D-Xyloside	$C_{20}H_{17}O_{10}$	417.30	Akkawi et al. (2019); Marques et al. (2016); Pinheiro et al. (2018)
29		Luteolin	$C_{15}H_{10}O_6$	286.24	Angamuthu et al. (2019); Trabelsi et al. (2020)
30		Luteolin-3′-*O*- β-D-glucoside	$C_{21}H_{20}O_{10}$	432.11	M. A. M. Nawwar et al. (1994)
31		Luteolin-4′-*O*- β-D-glucoside	$C_{21}H_{20}O_{10}$	432.11	Acquadro et al. (2020); M. A. M. Nawwar et al. (1994)
32		Luteolin-7-*O*-β-D-glucoside	$C_{21}H_{20}O_{11}$	448.40	Acquadro et al. (2020)
33		Luteolin-3′-*O*- β-D-Xyloside	$C_{20}H_{18}O_{10}$	418.09	M. A. M. Nawwar et al. (1994)
34		Naringenin 4′-methylether 7-*O*-α-L-arabinofuransyl (1-6)-β-D-glucoside	$C_{27}H_{32}O_{14}$	580.53	Chauhan and Chauhan (2001)
35		Pelargonidin	$C_{15}H_{11}O_5$	271.24	Swilam and Nematallah (2020)
36		Quercetin	$C_{15}H_{10}O_7$	302.24	Elfiky (2018); Ankita et al. (2015); Lakshminarayanashastry et al. (2019)
37		Quercetin 3-*O*-glucoside (Isoquercetin)	$C_{21}H_{20}O_{12}$	464.10	Acquadro et al. (2020); Angamuthu et al. (2019)
38		Quercetin-3-*O*-rutinoside (Rutin)	$C_{27}H_{30}O_{16}$	610.52	Elfiky (2018); Uysal et al. (2016); Lakshminarayanashastry et al. (2019)
39		Quercetin 3-rhamnoside (Quercitrin)	$C_{21}H_{20}O_{11}$	448.4	Mestry et al. (2020)
40	Phenolic acids and other organic acids	Benzoic acid	$C_7H_6O_2$	122.12	Uysal et al. (2016)
41		Caffeic acid	$C_9H_8O_4$	180.16	Farag et al. (2014)
42		Chlorogenic acid	$C_{16}H_{18}O_9$	345.31	Uysal et al. (2016); Farag et al. (2014)
43		Ferulic acid	$C_{10}H_{10}O_4$	194.18	Uysal et al. (2016)
44		Gallic acid	$C_7H_6O_5$	170.12	Akkawi et al. (2019); Elfiky (2018)
45		Maltol (volatile)	$C_6H_6O_3$	126.11	Bhinge et al. (2021)
46		*p*-Coumaric acid	$C_9H_8O_3$	164.05	Uysal et al. (2016)
47		*p*-Hydroxybenzoic acid	$C_7H_6O_3$	138.12	Elfiky (2018); Uysal et al. (2016)
48		Protocatechuic acid	$C_7H_6O_4$	154.12	Elfiky (2018)
49		Pyrogallic acid	$C_6H_6O_3$	126.11	Yu et al. (2017)
50		Quinic acid	$C_7H_{12}O_6$	192.17	Akkawi et al. (2019)
51		Syringic acid	$C_9H_{10}O_5$	198.17	Elfiky (2018)
52		Trihydroxybenzoic acid glycoside (Bergenin)	$C_{14}H_{16}O_9$	328.27	Mestry et al. (2020)
53		Vanillic acid	$C_8H_8O_4$	168.14	Elfiky (2018)
54	Simple galloyl derivatives	Brevifolin	$C_{12}H_8O_6$	248.19	Mahmoud A. M. Nawwar et al. (1994)
55		Brevifolin carboxylic acid	$C_{13}H_8O_8$	292.2	Akkawi et al. (2019); Mahmoud A. M. Nawwar et al. (1994)
56		Brevifolin carboxylic acid-10-monosulphate	$C_{13}H_7KO_{10}S$	394.25	Hussein et al. (1997)
57		Galloyl-HHDP-glucose	$C_{27}H_{15}O_{18}$	627.39	Pinheiro et al. (2019)
58		3,4,8,9,10-pentahydroxy-dibenzo [b,d]pyran-6-one	$C_{13}H_8O_7$	276.20	Mahmoud A. M. Nawwar et al. (1994)
59		1,2,3,4,6-Pent-*O*-galloyl-β-D-glucose	$C_{41}H_{32}O_{26}$	940.68	Mahmoud A. M. Nawwar et al. (1994)
60		1,2,4,6-Tetra-*O*-galloyl-β-D-glucose	$C_{34}H_{28}O_{22}$	788.57	Mahmoud A. M. Nawwar et al. (1994)
61		1,2,3-Tri-*O*-galloyl-β-D-glucose	$C_{27}H_{24}O_{18}$	636.47	Mahmoud A. M. Nawwar et al. (1994)
62		1,2,4-Tri-*O*-galloyl-β-D-glucose	$C_{27}H_{24}O_{18}$	636.47	Mahmoud A. M. Nawwar et al. (1994)
63		1,2,6-Tri-*O*-galloyl-β-D-glucose	$C_{27}H_{24}O_{18}$	636.47	Mahmoud A. M. Nawwar et al. (1994)

(*continued on next page*)

Table 1 (*continued*)

ID	Category	Compound Name	Formula	Molecular Weight	Reference
64		1,3,4-Tri-*O*-galloyl-β-D-glucose	$C_{27}H_{24}O_{18}$	636.47	Hussein et al. (1997)
65		1,4,6-Tri-*O*-galloyl-β-D-glucose	$C_{27}H_{24}O_{18}$	636.47	Mahmoud A. M. Nawwar et al. (1994)
66	Fatty acids	Arachidic acid	$C_{20}H_{40}O_2$	321.54	Uysal et al. (2015)
67		Behenic acid	$C_{22}H_{44}O_2$	340.59	Uysal et al. (2015)
68		Capric acid	$C_{10}H_{20}O_2$	172.27	Uysal et al. (2015)
69		Caprylic acid	$C_8H_{16}O_2$	144.21	Uysal et al. (2015)
70		Heneicosylic acid	$C_{21}H_{42}O_2$	326.55	Uysal et al. (2015)
71		Lauric acid	$C_{12}H_{24}O_2$	200.32	Uysal et al. (2015)
72		Linoleic acid	$C_{18}H_{32}O_2$	280.4	Elfiky (2018)
73		Margaric acid	$C_{17}H_{34}O_2$	270.45	Uysal et al. (2015)
74		Myristic acid	$C_{14}H_{28}O_2$	228.38	Uysal et al. (2015)
75		Palmitic acid	$C_{16}H_{32}O_2$	256.43	Uysal et al. (2015)
76		Pentadecylic acid	$C_{15}H_{30}O_2$	242.40	Uysal et al. (2015)
77		Stearic acid	$C_{18}H_{36}O_2$	284.48	Uysal et al. (2015)
78		Tridecylic acid	$C_{13}H_{26}O_2$	214.35	Uysal et al. (2015)
79		Undecylic acid	$C_{11}H_{22}O_2$	186.29	Uysal et al. (2015)
80	Sterols and terpenoids	Betulinic acid	$C_{30}H_{48}O_3$	456.70	Sanna et al. (2021)
81		2-Methyl-5-(1-methylethyl)-phenol (Carvacrol, a monoterpenic phenol)	$C_{10}H_{14}O$	150.22	Jebanesan et al. (2021)
82		Oleanolic acid	$C_{30}H_{48}O_3$	456.7	Sanna et al. (2021); Acquadro et al. (2020)
83		β-Sitosterol	$C_{29}H_{50}O$	414.71	Elbatanony et al. (2019)
84		Stigmasterol	$C_{29}H_{48}O$	412.70	Elfiky (2018)
85		Ursolic acid	$C_{30}H_{48}O_3$	456.70	Ramasamy et al. (2021)
86	Phenylethanoid	3-Hydroxytyrosol	$C_8H_{10}O_3$	154.16	Farag et al. (2014)
87		Verbascoside (Acetoside)	$C_{29}H_{36}O_{15}$	624.59	Lakshminarayanashastry et al. (2019)
88	Alkaloids	Cardenolide	$C_{23}H_{34}O_2$	342.51	Baranitharan et al. (2019)
89		4-Cyclopropylbenzaldehyde	$C_{10}H_{10}O$	146.18	Baranitharan et al. (2019)
90		Digoxigenin	$C_{23}H_{34}O_5$	390.51	Baranitharan et al. (2019)
91		2',6'-Dihydroxyacetophenone	$C_8H_8O_3$	152.15	Baranitharan et al. (2019)
92		3,5-Dimethylcyclohexanone	$C_8H_{14}O$	126.20	Baranitharan et al. (2019)
93		1-(2,5-dyihydroxy-phenyl)-pyridium chloride	$C_{11}H_{10}ClNO_2$	233.66	Baranitharan et al. (2019); M. A. M. Nawwar et al. (1994)
94		Methyl 4-piperidineacetate	$C_8H_{15}NO_2$	157.21	Baranitharan et al. (2019)
95		n-Boc-4-piperidineacetaldehyde	$C_{12}H_{21}NO_3$	227.30	Baranitharan et al. (2019)
96	Vitamins and minerals	Calcium	Ca	40.08	Elfiky (2018)
97		Folic acid (Vitamin B_9)	$C_{19}H_{19}N_7O_6$	441.4	Mestry et al. (2020)
98		Iron	Fe	55.85	Elfiky (2018)
99		Phosphorus	P	30.97	Elfiky (2018)
100		Potassium	K	39.10	Elfiky (2018)
101		Sodium	Na	22.99	Elfiky (2018)
102		Vitamin A	–	–	Elfiky (2018)
103		Vitamin E	–	–	Elfiky (2018)
104	Other compounds	Coniferyl 9-*O*-[β-D-apiofuransyl-(1-6)]-*O*-β-D-glucopyranside	$C_{21}H_{30}O_{12}$	474.46	Swilam and Nematallah (2020)
105		Dulcitol (Galactitol)	$C_6H_{14}O_6$	182.17	Mestry et al. (2020)
106		12-Hydroperoxyicosatetraenoic acid (12(*S*)-Hpete)	$C_{20}H_{32}O_4$	336.5	Fellah et al. (2020)
107		Loganin (an iridoid monoterpenoid)	$C_{17}H_{26}O_{10}$	390.4	Mestry et al. (2020)
108		2-Methyl-pyran-4-one-3-*O*-β-D-glucopyranoside	$C_{12}H_{16}O_8$	288.25	Balwani et al. (2011)
109		14-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	Jebanesan et al. (2021)
110		18-Oxooleate	$C_{18}H_{32}O_3$	296.4	Fellah et al. (2020)
111		Solasodine-3-*O*-β-d-glucopyranoside	$C_{33}H_{54}NO_7$	576.8	Fellah et al. (2020)

HHDP: Hexahydroxydiphenic acid.

Compounds in each category are sorted alphabetically.

Acquadro, S., Civra, A., Cagliero, C., Marengo, A., Rittà, M., Francese, R., … Lembo, D. (2020). *Punica granatum* leaf ethanolic extract and ellagic acid as inhibitors of Zika virus infection. *Planta Medica, 86*(18), 1363-1374. doi:10.1055/a-1232-5705

Akkawi, M., Abu-Lafi, S., & Abu-Remeleh, Q. (2019). Phytochemical screening of Pomegranate juice, peels, leaves and membranes water extracts and their effect on β-hematin formation, a comparative study. *Pharm. Pharmacol. Int. J, 7*, 193-200. doi:10.15406/ppij.2019.07.00251

Angamuthu, D., Purushothaman, I., Kothandan, S., & Swaminathan, R. (2019). Antiviral study on *Punica granatum* L., *Momordica charantia* L., *Andrographis paniculata* Nees, and *Melia azedarach* L., to Human Herpes Virus-3. *European Journal of Integrative Medicine, 28*, 98-108. doi:https://doi.org/10.1016/j.eujim.2019.04.008

Ankita, P., Deepti, B., & Nilam, M. (2015). Flavonoid rich fraction of Punica granatum improves early diabetic nephropathy by ameliorating proteinuria and disturbed glucose homeostasis in experimental animals. *Pharmaceutical Biology, 53*(1), 61-71. doi:10.3109/13880209.2014.910533

Balwani, S., Nandi, D., Jaisankar, P., & Ghosh, B. (2011). 2-Methyl-pyran-4-one-3-O-β-d-glucopyranoside isolated from leaves of *Punica granatum* inhibits the TNFα-induced cell adhesion molecules expression by blocking nuclear transcription factor-κB (NF-κB). *Biochimie, 93*(5), 921-930. doi:https://doi.org/10.1016/j.biochi.2011.01.010

Baranitharan, M., Tamizhazhagan, V., Kovendan, K., & Senthilmurugan, S. (2019). *Punica granatum*-based green ethanolic extract as highly effective and eco-friendly larvicide, repellent against medically important mosquito vectors. *Entomol. Appl. Sci. Lett, 6*, 33-41.

Bhinge, S. D., Bhutkar, M. A., Randive, D. S., Wadkar, G. H., Todkar, S. S., Savali, A. S., & Chittapurkar, H. R. (2021). Screening of hair growth promoting activity of *Punica granatum* L. (pomegranate) leaves extracts and its potential to exhibit antidandruff and anti-lice effect. *Heliyon, 7*(4), e06903. doi:https://doi.org/10.1016/j.heliyon.2021.e06903

Chauhan, D., & Chauhan, J. S. (2001). Flavonoid diglycoside from *Punica granatum*. *Pharmaceutical Biology, 39*(2), 155-157. doi:10.1076/phbi.39.2.155.6254

El-Toumy, S. A. A., & Rauwald, H. W. (2002). Two ellagitannins from *Punica granatum* heartwood. *Phytochemistry, 61*(8), 971-974. doi:https://doi.org/10.1016/S0031-9422(02)00435-1

Elbatanony, M. M., El-Feky, A. M., Hemdan, B. A., & Azab El-Liethy, M. (2019). Assessment of the antimicrobial activity of the lipoidal and pigment extracts of *Punica granatum* L. leaves. *Acta Ecologica Sinica, 39*(1), 89-94. doi:https://doi.org/10.1016/j.chnaes.2018.05.003
Elfiky, A. M. A. (2018). A pharmacognosical study of *Punica granatum* var. nana cultivated in Egypt. *CU Theses*.
Farag, R., Abdel-Latif, M. S., Emam, S., & Tawfeek, L. (2014). Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. *Agric Soil Sci, 1*(6), 86-93. Retrieved from http://www.landmarkresearchjournals.org/lrjass/index.php
Fellah, B., Rocchetti, G., Senizza, B., Giuberti, G., Bannour, M., Ferchichi, A., & Lucini, L. (2020). Untargeted metabolomics reveals changes in phenolic profile following *in vitro* large intestine fermentation of non-edible parts of *Punica granatum* L. *Food Research International, 128*, 108807. doi:10.1016/j.foodres.2019.108807
Hussein, S. A. M., Barakat, H. H., Merfort, I., & Nawwar, M. A. M. (1997). Tannins from the leaves of *Punica granatum*. *Phytochemistry, 45*(4), 819-823. doi:Doi 10.1016/S0031-9422(96)00888-6
Jebanesan, A., Baranitharan, M., Kovendan, K., & Avery, P. B. (2021). Impact of *Punica granatum*-based green larvicide on the predation rate of *Polypedates cruciger* for the control of mosquito vectors, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *International Journal of Tropical Insect Science, 41*(2), 1075-1085. doi:10.1007/s42690-020-00293-7
Lakshminarayanashastry, V. G., Venkatanarasappa, V. M., & Lingeswara, P. N. B. (2019). Methanolic leaf extract of *Punica granatum* attenuates ischemia-reperfusion brain injury in Wistar rats: potential antioxidant and anti-inflammatory mechanisms. *Iranian journal of basic medical sciences, 22*(2), 187.
Li, Y., Yang, F., Zheng, W., Hu, M., Wang, J., Ma, S., … Yin, W. (2016). Punica granatum (pomegranate) leaves extract induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in non-small cell lung cancer in vitro. *Biomedicine & Pharmacotherapy, 80*, 227-235. doi:https://doi.org/10.1016/j.biopha.2016.03.023
Marques, L. C., Pinheiro, A. J., Araújo, J. G., de Oliveira, R. A., Silva, S. N., Abreu, I. C., … Silbiger, V. N. (2016). Anti-inflammatory effects of a pomegranate leaf extract in LPS-induced peritonitis. *Planta Medica, 82*(17), 1463-1467. doi:10.1055/s-0042-108856
Mestry, S., Gawali, N., Pai, S., Gursahani, M., Dhodi, J., Munshi, R., & Juvekar, A. (2020). *Punica granatum* improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. *Journal of Ayurveda and integrative medicine, 11*(1), 16-23. doi:10.1016/j.jaim.2017.09.006
Nawwar, M. A. M., Hussein, S. A. M., & Merfort, I. (1994). Leaf phenolics of *Punica granatum*. *Phytochemistry, 37*(4), 1175-1177. doi:Doi 10.1016/S0031-9422(00)89552-7
Nawwar, M. A. M., Hussein, S. A. M., & Merfort, I. (1994). NMR spectral analysis of polyphenols from *Punica granatum*. *Phytochemistry, 36*(3), 793-798. doi:https://doi.org/10.1016/S0031-9422(00)89820-9
Nostro, A., Guerrini, A., Marino, A., Tacchini, M., Di Giulio, M., Grandini, A., … Saraçoğlu, H. T. (2016). *In vitro* activity of plant extracts against biofilm-producing food-related bacteria. *International Journal of Food Microbiology, 238*, 33-39. doi:https://doi.org/10.1016/j.ijfoodmicro.2016.08.024
Orgil, O., Schwartz, E., Baruch, L., Matityahu, I., Mahajna, J., & Amir, R. (2014). The antioxidative and anti-proliferative potential of non-edible organs of the pomegranate fruit and tree. *LWT - Food Science and Technology, 58*(2), 571-577. doi:10.1016/j.lwt.2014.03.030
Pinheiro, A. J. M. C. R., Gonçalves, J. S., Dourado, Á. W. A., de Sousa, E. M., Brito, N. M., Silva, L. K., … Lima-Neto, L. G. (2018). *Punica granatum* L. leaf extract attenuates lung inflammation in mice with acute lung injury. *Journal of immunology research, 2018*, 6879183. doi:10.1155/2018/6879183
Pinheiro, A. J. M. C. R., Mendes, A. R. S., Neves, M. D. F. d. J., Prado, C. M., Bittencourt-Mernak, M. I., Santana, F. P. R., … Lima-Neto, L. G. (2019). Galloyl-hexahydroxydiphenoyl (HHDP)-glucose isolated from *Punica granatum* L. leaves protects against lipopolysaccharide (LPS)-induced acute lung injury in BALB/c mice. *Frontiers in immunology, 10*(1978). doi:10.3389/fimmu.2019.01978
Pottathil, S., Nain, P., Morsy, M. A., Kaur, J., Al-Dhubiab, B. E., Jaiswal, S., & Nair, A. B. (2020). Mechanisms of Antidiabetic Activity of Methanolic Extract of Punica granatum Leaves in Nicotinamide/Streptozotocin-Induced Type 2 Diabetes in Rats. *Plants, 9*(11), 1609. Retrieved from https://www.mdpi.com/2223-7747/9/11/1609
Ramasamy, N., Periyanayagam, K., & Sugithra, B. (2021). Pomegranate (*Punica granatum*. Linn. var. Ganesh) leaf extracts ameliorates neurotoxicity in transgenic *Drosophila* expressing human amyloid pathology. *Indian Journal of Pharmaceutical and Biological Research, 9*(01), 1-7. doi:https://doi.org/10.30750/ijpbr.9.1.1
Sanna, C., Marengo, A., Acquadro, S., Caredda, A., Lai, R., Corona, A., … Esposito, F. (2021). *In vitro* anti-HIV-1 reverse transcriptase and integrase properties of *Punica granatum* L. leaves, bark, and peel extracts and their main compounds. *Plants, 10*(10), 2124. Retrieved from https://www.mdpi.com/2223-7747/10/10/2124
Swilam, N., & Nematallah, K. A. (2020). Polyphenols profile of pomegranate leaves and their role in green synthesis of silver nanoparticles. *Scientific Reports, 10*(1), 14851. doi:10.1038/s41598-020-71847-5
Tanaka, T., Nonaka, G.-I., & Nishioka, I. (1985). Punicafolin, an ellagitannin from the leaves of *Punica granatum*. *Phytochemistry, 24*(9), 2075-2078. doi:https://doi.org/10.1016/S0031-9422(00)83125-8
Toda, K., Ueyama, M., Tanaka, S., Tsukayama, I., Mega, T., Konoike, Y., … Suzuki-Yamamoto, T. (2020). Ellagitannins from *Punica granatum* leaves suppress microsomal prostaglandin E synthase-1 expression and induce lung cancer cells to undergo apoptosis. *Bioscience, Biotechnology, and Biochemistry, 84*(4), 757-763. doi:10.1080/09168451.2019.1706442
Trabelsi, A., El Kaibi, M. A., Abbassi, A., Horchani, A., Chekir-Ghedira, L., & Ghedira, K. (2020). Phytochemical study and antibacterial and antibiotic modulation activity of *Punica granatum* (pomegranate) leaves. *Scientifica, 2020*, 8271203. doi:10.1155/2020/8271203
Uysal, S., Zengin, G., Aktumsek, A., & Karatas, S. (2015). Fatty acid composition, total sugar content and anti-diabetic activity of methanol and water extracts of nine different fruit tree leaves collected from Mediterranean region of Turkey. *International Journal of Food Properties, 18*(10), 2268-2276. doi:10.1080/10942912.2014.973964
Uysal, S., Zengin, G., Aktumsek, A., & Karatas, S. (2016). Chemical and biological approaches on nine fruit tree leaves collected from the Mediterranean region of Turkey. *Journal of functional foods, 22*, 518-532. doi:https://doi.org/10.1016/j.jff.2016.02.006
Yu, X., Wang, X.-P., Lei, F., Jiang, J.-F., Li, J., Xing, D.-M., & Du, L.-J. (2017). Pomegranate leaf attenuates lipid absorption in the small intestine in hyperlipidemic mice by inhibiting lipase activity. *Chinese Journal of Natural Medicines, 15*(10), 732-739. doi:https://doi.org/10.1016/S1875-5364(17)30104-8

ethyl acetate, dichloromethane, ethanol, and methanol, PGL had the highest TFC in ethanolic extracts and the most anthocyanins in hexane extracts (Bekir, Mars, Souchard, & Bouajila, 2013). The ethanol and ethyl acetate fractions of Egyptian PGLE contained various anthocyanins and their derivatives, such as cyanidin pentoside, procyanidin dimer, pelargonidin, and delphinidin dihexoside (Swilam & Nematallah, 2020). In contrast, aqueous PGLE had cyanidin-3,5-diglucoside and kaempferol 3-O-b-D-xyloside (Akkawi et al., 2019).

Research indicates that PGL has a higher TFC compared to many medicinal or fruit plants or even other parts of the pomegranate. The ethanolic PGLE had the highest TFC (237 μg QE/g) among ten Thai edible and medicinal plants (Kaewnarin et al., 2014). When compared to non-edible pomegranate parts, PGL' TFC (63.89 mg QE/g) was more than the stem and seed but less than the flower and peel (Rummun, Somanah, Ramsaha, Bahorun, & Neergheen-Bhujun, 2013). The Nana variety's ethanolic PGLE had a higher TFC than the rind (Elfiky, 2018). The Nabli PGL variety had more flavones in its leaves and flowers (Fellah et al., 2018; Fellah et al., 2020). In the Tounsian variety, PGL had the highest TFC compared to the flower, peel, and juice (Amri et al., 2017).

2.3. Tannins

Pomegranate leaf (PGL) is abundant in hydrolyzable tannins (HTs),

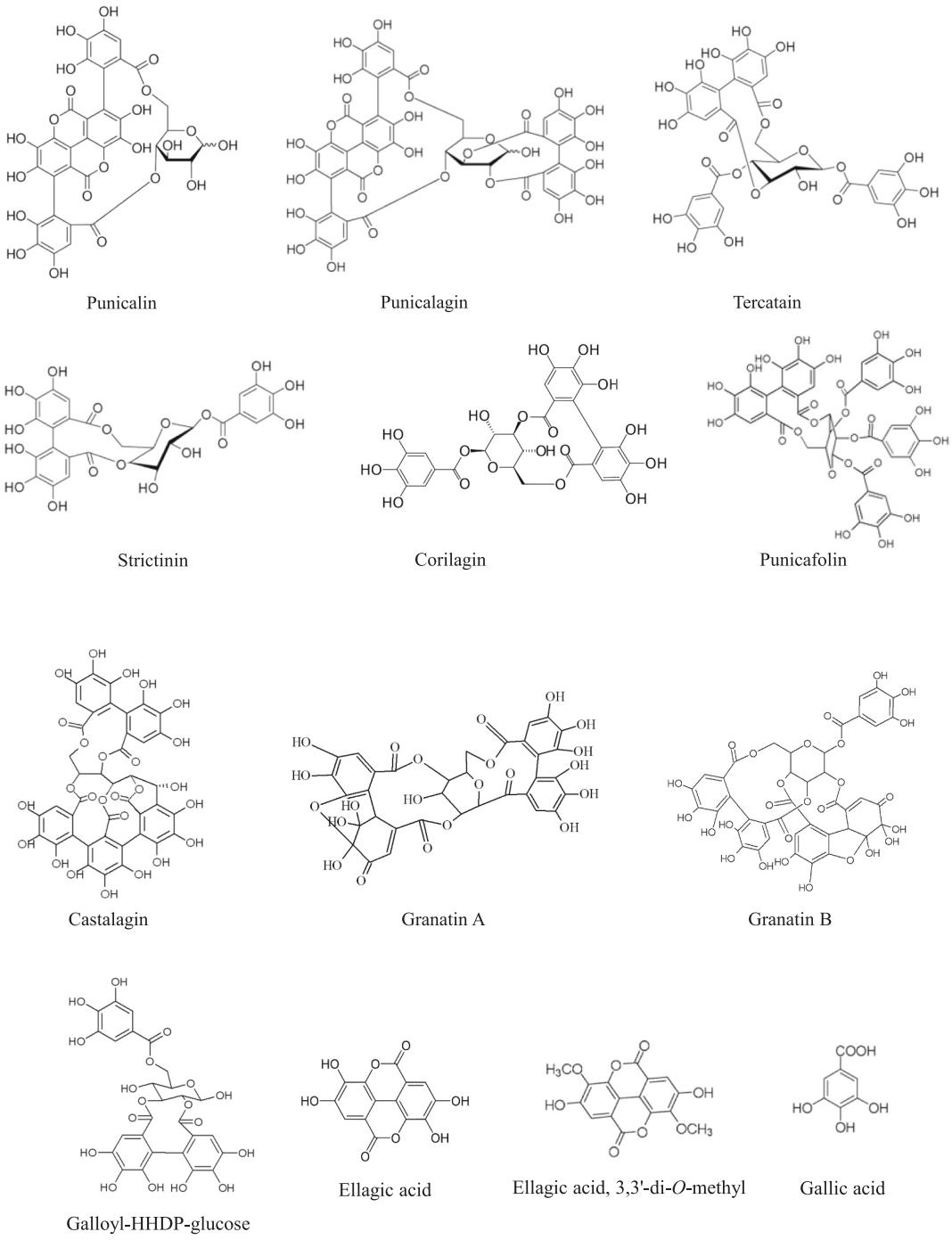

Fig. 1. Chemical structures of major compounds present in pomegranate leaves. Abbreviation: HHDP – hexahydroxydiphenoyl.

which include both gallotannins (GTs) and ellagitannins (ETs), as well as condensed tannins (CTs), as detailed in Table 1. The total tannin content (TTC) of hydroalcoholic PGLE from India was found to be 22% (*w*/w) using the hide-powder method (Ankita et al., 2015). LC-MS/MS analysis of methanolic PGLE revealed a concentration of 11.65 μg/mg of PGG, a type of GTs that plays a role in ETs formation (Viswanatha, Venkatarangana, & Prasad, 2019). Brazilian PGLE, when analyzed, showed the presence of compounds like galloyl-HHDP-glucose, ETs (including punicalin and castalagin derivatives), and ellagic acid derivatives such as ellagic acid rhamnoside and 3,3′-di-*O*-methylellagic acid in its hydroalcoholic extract and ethyl acetate fraction (Marques et al., 2016; Pinheiro et al., 2018; Pinheiro et al., 2019). Japanese PGLE, notably, contained tannins like granatin A, granatin B, and geraniin, predominantly in its ethyl acetate and water-saturated n-butanol extracts (Toda et al., 2020).

Egyptian and Palestinian PGL, when examined, revealed a diverse array of tannins in their hydroalcoholic and hot water extracts, respectively. These primarily included galloyl derivatives and ellagic acid derivatives, such as corilagin, brevifolin carboxylic acid, ellagic acid hexoside, and eschweilenol C (Acquadro et al., 2020; Akkawi et al., 2019; Swilam & Nematallah, 2020). In the Israeli Wonderful accession of PGLE, the concentrations of punicalagin and gallagic acid were notably higher than ellagic acid and gallic acid (Orgil et al., 2014). This difference is consistent with the role of ellagic and gallic acids as

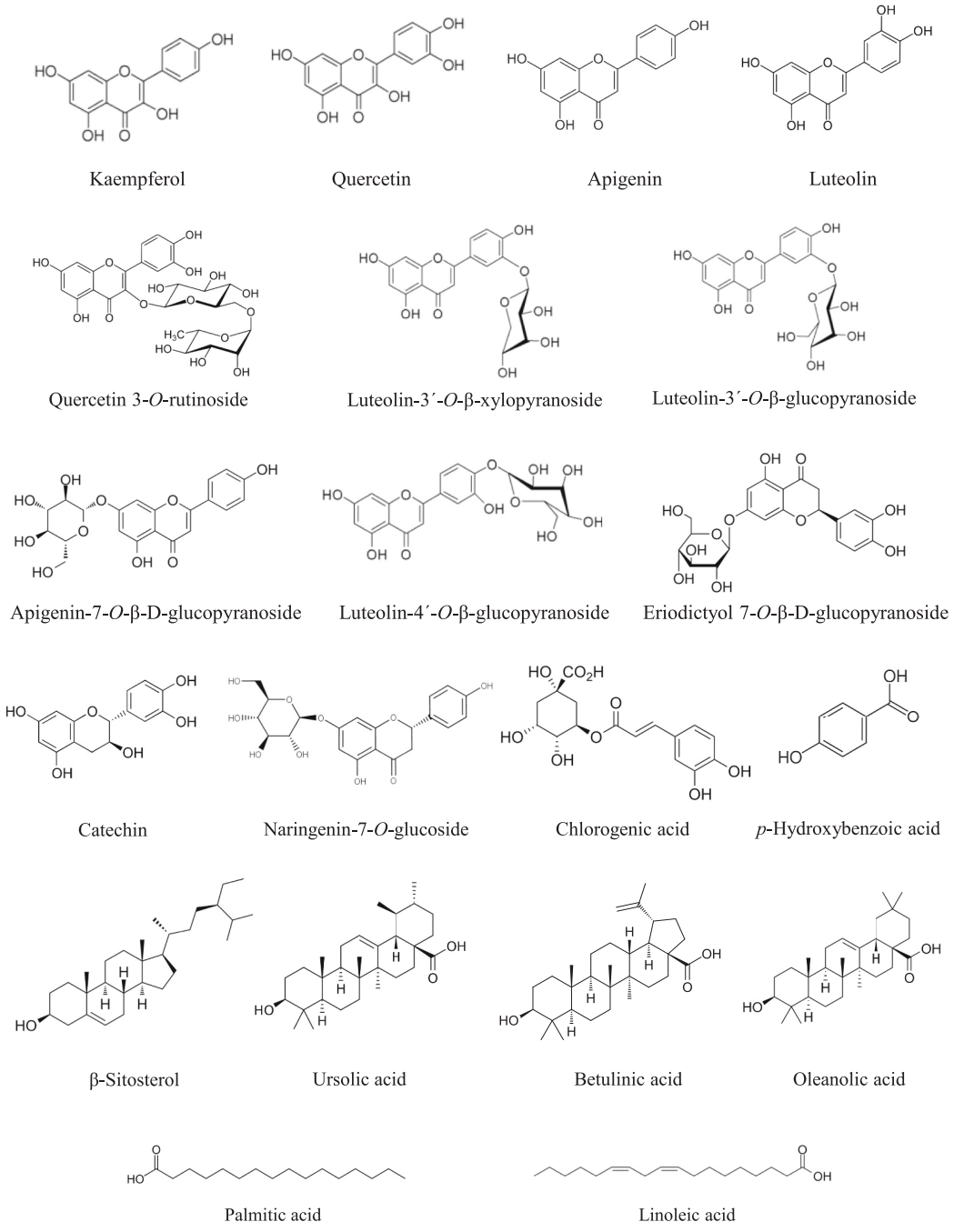

Fig. 1. (*continued*).

precursors for ETs like punicalagin (Ono, Bandaranayake, & Tian, 2012). Other tannins, such as strictinin and corilagin, were identified in lyophilized PGLE from India (Angamuthu, Purushothaman, Kothandan, & Swaminathan, 2019), while tannic acid was reported in Chinese PGL (X. Yu et al., 2017). The Tunisian soft-seed pomegranate's ethanolic PGLE contained punicalagin and ellagic acid at 39.6 and 32 mg/g, respectively (Li et al., 2016).

Factors like seasonal changes, variety, and extraction methods influence the concentrations of various tannins (Xiang et al., 2008). Zhang et al. (2010) recommended extracting PGL post-August, as the bioactive compound content increased until September's end. The Tounsi variety from Tunisia's Mahdia region had a higher TTC (17 mg TAE/g) than the Nana variety from Moknine, which had 13 mg TAE/g (Amri et al., 2017). Among different extraction techniques, aqueous PGLE had the highest TTC (125.92 mg TAE/100 g), followed by ethanol, TOF, ethyl acetate, chloroform, and hexane extracts (Trabelsi et al., 2020).

Comparative studies suggest that the TTC in PGL is often higher than in other medicinal plants or different parts of the pomegranate. For instance, hydro-methanolic PGLE had the highest TTC (99.20 mg ECE/g) when compared to renowned food and medicinal plants like peppermint, sage, rosemary, rue, olive leaves, and parsley (M. Yu, Gouvinhas, Rocha, & Barros, 2021). Additionally, the aqueous PGLE's HTs content surpassed that in the seed, flower, and peel, but the methanolic PGLE's HTs content was on par with the peel (Elfalleh et al., 2012).

2.4. Other compounds in pomegranate leaves

Pomegranate Leaf Extracts (PGLE) contain a diverse range of bioactive compounds, including phenols (7.76 mg/g), alkaloids (6.18 mg/g), and terpenoids (1.82 mg/g) as predominant components (Durgadevi et al., 2018; Ramamurthy & Marueen, 2018). Notably, the Nabli pomegranate variety exhibited high levels of tyrosols (5.3 g/kg) (Fellah et al., 2018; Fellah et al., 2020). Ethanolic PGLE revealed triterpenoids like oleanolic acid and ursolic acid, with the latter being significant in the Indian Ganesh cultivar (Acquadro et al., 2020; Sanna et al., 2021). PGL is rich in saturated fatty acids (51.91%), particularly palmitic acid, and unsaturated fatty acids, led by linolenic acid, which make up 37.78% (Elfiky, 2018). Additionally, PGL contains essential amino acids (5.37–10.89 g/100 g), with leucine and valine being the most abundant (Elfiky, 2018). The Egyptian Nana variety's ethyl acetate fraction showed higher lipoidal matter in leaves than peels, with stigmasterol and β-amyrin being primary lipoidal compounds (Elfiky, 2018). Carotenoids such as lutein and β-carotene, chlorophylls, ergosterol, and α-tocopherol were also identified in various PGLE extracts (Amri et al., 2017; Elbatanony, El-Feky, Hemdan, & Azab El-Liethy, 2019). In PGL, the unsaponifiable matter yield was higher (40.71%) compared to peels (24.17%) (Elfiky, 2018). Alcoholic PGLE of Hungarian accession contained volatile compounds like maltol (36.23%) (Bhinge et al., 2021), while β-sitosterol (5.77%) was the main sterol in PGL, with various steroids such as cardenolide and digoxigenin identified in Indian PGLE (Baranitharan, Tamizhazhagan, Kovendan, & Senthilmurugan, 2019; Elfiky, 2018). The primary minerals in PGL include potassium (2.6 g/100 g), sodium (0.32 g/100 g), and calcium (0.3 g/100 g), with iron and phosphorus present in lower amounts (Elfiky, 2018). PGLE also contains specialized compounds like coumarin (Durgadevi et al., 2018; Farag et al., 2014), acteoside (Viswanatha et al., 2019), and feruloyl coniferin (Swilam & Nematallah, 2020). A unique anti-inflammatory compound, 2-methyl-pyran-4-one-3-O-β-D-glucopyranoside, was identified in hydroalcoholic PGLE (Balwani, Nandi, Jaisankar, & Ghosh, 2011). Additionally, supercritical fluid extracts of PGL revealed compounds like eicosanol and squalene (Cavalcanti, Navarro-Díaz, Santos, Rostagno, & Meireles, 2012).

3. Biological activities and human health of pomegranate leaves

3.1. Antioxidant activity in reducing oxidative stress and preventing chronic diseases

Pomegranate leaves (PGL) are a rich source of natural antioxidants, with their antioxidant capacity (AOC) attributed to a variety of phenolics including phenolic acids, flavonoids, and tannins. These components vary significantly depending on the extraction methods, solvents used, and the specific pomegranate cultivars grown under different geographical conditions. Studies (Kaneria et al., 2012; Ouédraogo et al., 2021) have demonstrated that different extraction techniques and solvents can greatly influence the AOC of PGL. For instance, Kaneria et al. (2012) found that successive extraction using acetone was particularly effective in extracting antioxidants from Indian PGL, showing higher DPPH and superoxide anion radical scavenging capacities, as well as ferric reducing antioxidant power (FRAP), compared to pomegranate stem. The correlation between the total phenolic content (TPC) and total flavonoid content (TFC) of PGL and their AOC has been highlighted in studies like Zhang et al. (2010), who also emphasized the significance of seasonal changes on the TPC and TFC in Chinese PGL, suggesting the optimal harvesting time to maximize the biological yield and active compounds. Further research by Ouédraogo et al. (2021) and Viswanatha et al. (2019)) has revealed that the phenolic composition and AOC of PGL can vary between different cultivars. For example, the Ganesh cultivar showed higher AOC compared to the Kesar cultivar, which was linked to its higher TPC and TFC.

Comparative studies (Kolar et al., 2021; Rummun et al., 2013) of non-edible and edible sections of pomegranate have consistently shown that the non-edible parts, including the leaves, have higher levels of phenolics and AOC (Table S4). This finding positions PGL as a promising alternative to more commonly used parts like the peel and seeds. Furthermore, PGL have demonstrated higher AOC than many well-known medicinal plants. M. Yu et al. (2021) found that Portuguese hydro-methanolic PGL extracts exhibited significantly higher antioxidant activity compared to other medicinal species like sage and rosemary.

The substantial AOC of PGL, as evidenced by the various *in vitro* studies (Table S5), underscores their potential in reducing oxidative stress and preventing chronic diseases. These potentials may involve several key mechanisms (Fig. 2), primarily driven by their rich content of antioxidant phytochemicals.

Polyphenols in pomegranate leaves donate hydrogen atoms or electrons to neutralize free radicals, as well as chelate metal ions, preventing the formation of harmful radicals (R. P. Singh, Chidambara Murthy, & Jayaprakasha, 2002). Compounds in pomegranate leaves can influence the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Bhandary, Bhat, & Bekal, 2012). Flavonoids and other phenolic compounds can inhibit enzymes that generate ROS, such as xanthine oxidase and NADPH oxidase (Negi, Jayaprakasha, & Jena, 2003). Phytochemicals in pomegranate leaves can activate cellular antioxidant defense systems. This includes upregulating the expression of genes encoding for antioxidant enzymes and enhancing the production of non-enzymatic antioxidants like glutathione (Jurenka, 2008). Tannins and other phenolic compounds in pomegranate leaves can prevent the peroxidation of lipids, thereby protecting cell membranes (Seeram et al., 2005). Some compounds in pomegranate leaves may interact with cellular signaling pathways involved in oxidative stress responses, such as the NF-κB pathway (Haseeb, Khan, Ashruf, & Haqqi, 2017). Antioxidants in pomegranate leaves can directly interact with DNA and proteins, protecting these crucial biomolecules from oxidative damage (Kasai, Yoshimura, Koga, Arii, & Kawasaki, 2006).

By tapping into the rich antioxidant profile of PGL, there is an opportunity to develop novel dietary supplements and medicinal products, contributing to a more sustainable and health-conscious future.

3.2. Enhanced anti-inflammatory profile of pomegranate leaf extracts

3.2.1. Diverse solvent extraction benefits

Pomegranate leaf extract (PGLE) has been established as a versatile anti-inflammatory agent, with its efficacy influenced by the extraction solvent. This variability is attributed to the differential extraction of antioxidants, especially phenolic compounds, which are pivotal in modulating inflammatory responses (Bekir et al., 2013). Both *in vitro* and *in vivo* studies have corroborated the extract's potency in reducing inflammation.

3.2.2. In Vivo anti-inflammatory and anti-nociceptive effects

Indian hydroalcoholic PGLE has demonstrated a dose-responsive attenuation of inflammation in both acute (carrageenan-induced paw edema) and chronic (cotton pellet-induced granuloma) models. Additionally, it exhibited anti-nociceptive properties in an acetic acid-induced pain model in mice, highlighting its potential for pain management (Salwe & Sachdev, 2014). The ethyl acetate fraction of ethanolic PGLE has been particularly noted for its superior efficacy in diminishing inflammatory markers such as TNF-α and IL-6 in a diabetic rat model (Elfiky, 2018). Methanolic PGLE also conferred renal protection in a gentamicin-induced oxidative stress model and offered neuroprotection against ischemia/reperfusion-induced brain injury by modulating brain cytokine levels (S. Mestry et al., 2020; Viswanatha et al., 2019).

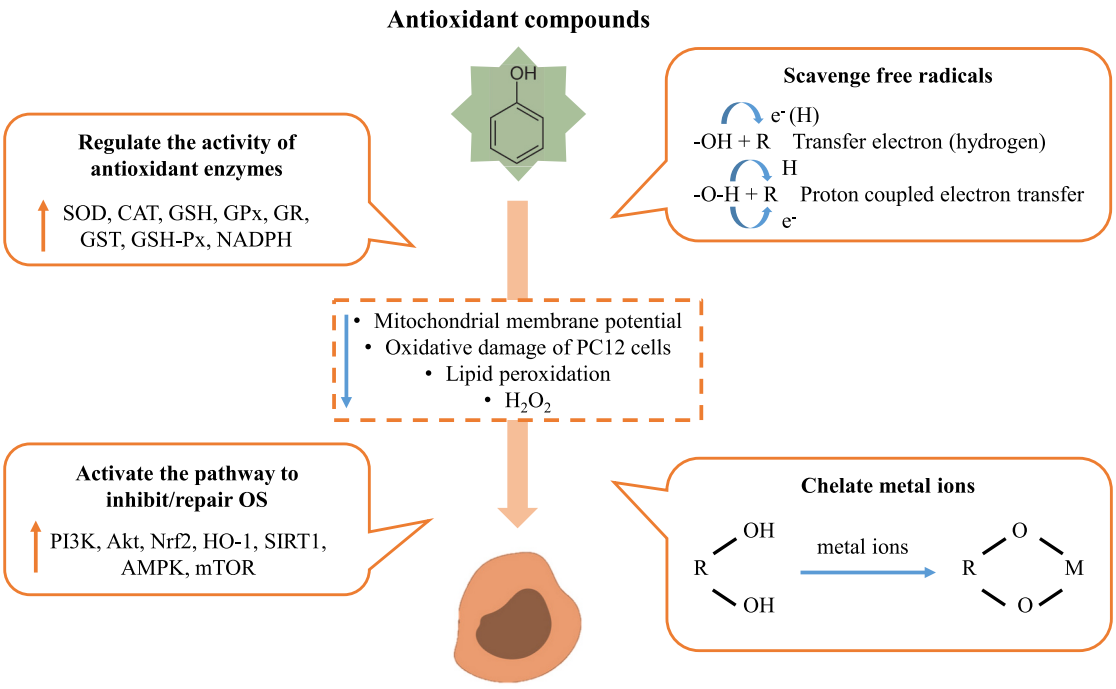

Fig. 2. Possible mechanisms of antioxidant activity of pomegranate leaves. Abbreviations: SOD – superoxide dismutase; CAT – catalase; GSH – glutathione; GPx – glutathione peroxidase; GR – glutathione reductase; GST – glutathione S-transferase; GSH-Px – seleno-dependent glutathione peroxidase; NADPH – nicotinamide adenine dinucleotide phosphate; OS – oxidative stress; PI3K – phosphoinositide 3-kinase; Akt – protein kinase B; Nrf2 – nuclear factor erythroid 2-related factor 2; HO-1 – heme oxygenase-1; SIRT1 – sirtuin 1; AMPK – AMP-activated protein kinase; mTOR – mammalian target of rapamycin; R – phenolic acids or flavonoids; M – metal ions.

3.2.3. *Targeted anti-inflammatory compounds*

Investigations into specific PGLE constituents, such as ellagitannins, have revealed their role in inducing apoptosis in lung cancer cells and concurrently reducing inflammatory mediators like TNFα, iNOS, and mPGES-1 (Toda et al., 2020). The compound galloyl-HHDP-glucose, isolated from the ethyl acetate fraction, has shown promise against LPS-induced acute lung injury in mice (Pinheiro et al., 2018; Pinheiro et al., 2019).

3.2.4. *Molecular mechanisms in cell lines and enzymatic inhibition*

Pomegranate leaves exhibit multifaceted anti-inflammatory effects through several mechanisms (Fig. 3). At the cellular level, *n*-butanol PGLE has been shown to inhibit the activation and translocation of NF-κB and reduce TNF-α-induced expression of cell adhesion molecules on human umbilical vein endothelial cells (HUVEC), suggesting a blockade of inflammatory cell migration (Balwani et al., 2011). The anti-inflammatory prowess of PGLE was further evidenced by its superior performance over pomegranate flower extracts in the human red blood cell (HRBC) membrane stability assay, even outshining the reference standard (Gheith & El-Mahmoudy, 2017). Both ethanolic and methanolic extracts displayed significant anti-inflammatory activity against 5-lipoxygenase, on par with the reference drug nordihydroguaiaretic acid (Bekir et al., 2013).

The novelty of PGL's anti-inflammatory effects lies in its enzyme-specific inhibition and pathway modulation, which could lead to more targeted and effective treatments. The applications extend to managing pain, chronic inflammation, and potentially treating neurodegenerative and renal diseases, positioning PGL as a valuable resource for natural health product development and pharmacotherapy.

3.3. *Innovative anti-cancer potential of pomegranate leaves*

Oxidative stress and inflammatory response play crucial roles in cancer progression. This review delves into pioneering research on the anti-cancer properties of pomegranate leaf extract (PGLE), emphasizing its novel applications in inhibiting various cancer cell types. Among the most studied cancers, liver cancer has shown promising results, with silver nanoparticles (AgNPs) synthesized with aqueous PGLE demonstrating dose-responsive potential in curbing human liver cancer cells (HepG2) with significant efficacy (Saratale et al., 2018). Indian PGLE's hepatoprotective qualities were demonstrated through the upregulation of total protein and the downregulation of liver damage markers in toxin-induced hepatotoxicity models (Kumar et al., 2021). Additionally, ethanolic PGLE, particularly when combined with gallic acid, has been effective in reversing liver dysfunction and mitigating oxidative stress in dietary-induced liver damage (Rao & Krishnamurthy, 2019). In the context of colon cancer, petroleum ether extracts of pomegranate leaves have exhibited pronounced cytotoxicity against colon cancer cells (HCT-116), surpassing effects on other plant parts (Elfiky, 2018), while methanolic PGLE has shown strong cytotoxic influence against HT29 colon cancer cells (Balamurugan, Karuppasamy, Sivaraj, Saraswathi, & Arumugam, 2021). For breast cancer, the Tunisian Gabsi variety of methanolic PGLE has been highly cytotoxic against MCF-7 breast cancer cell lines, indicating robust anti-cancer capability (Balamurugan et al., 2021; Bekir et al., 2013). Ethanol extracts of pomegranate leaf and peel also demonstrated significant cytotoxic activity against MCF-7 cells, with variations observed across different extraction solvents and methods (Orgil et al., 2014). Additionally, ethanolic Tunisian PGLE has been proposed as a chemotherapeutic agent for non-small cell lung cancer (NSCLC) cell lines, impeding cell migration and invasion, arresting cell cycle progression, and inducing apoptosis (Li et al., 2016). Ellagitannins from PGLE have been reported to induce apoptosis in lung cancer cells (A549), suggesting a mechanism involving the down-regulation of inflammatory mediators (Toda et al., 2020).

Beyond these well-studied cancers, pomegranate leaf extracts have also shown potential against gynecological, hematological, and prostate

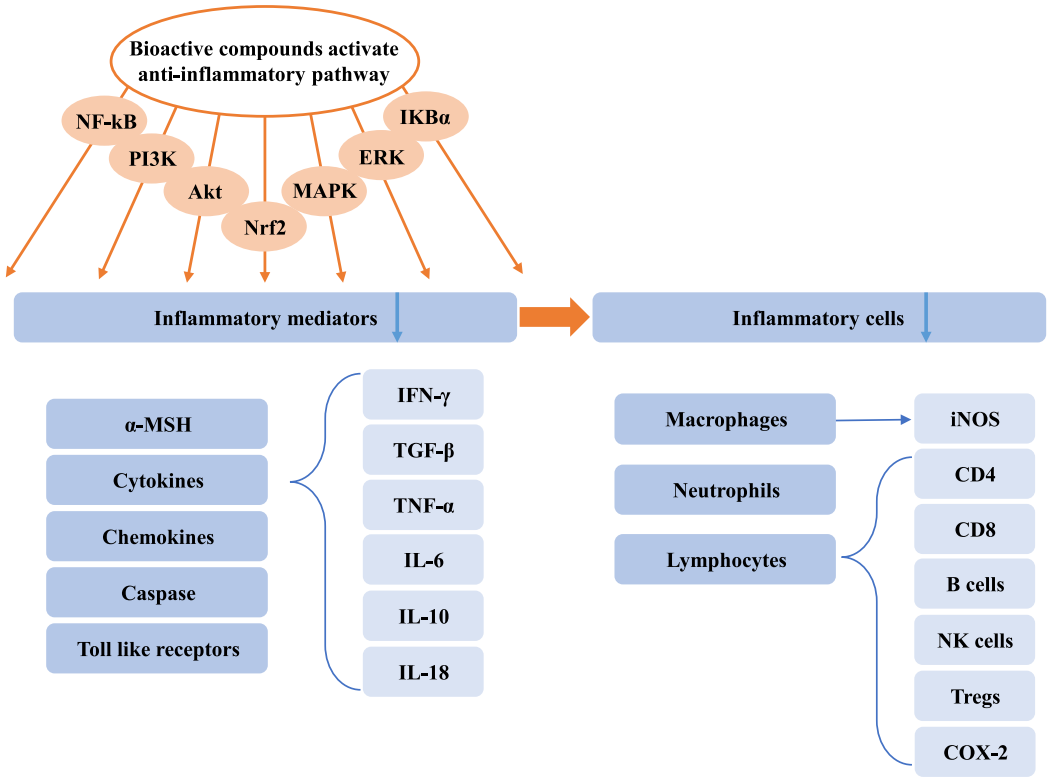

Fig. 3. Possible mechanisms of anti-inflammatory profile of pomegranate leaves. Abbreviations: NF-kB – nuclear transcription factor kappa B; PI3K – phosphoinositide 3-kinase; Akt – protein kinase B; Nrf2 – nuclear factor erythroid 2-related factor 2; MAPK – mitogen-activated protein kinase; ERK – extracellular-signal-regulated kinase; IKBα – I Kappa B kinase alpha; α-MSH – alpha melanocyte-stimulating hormone; IFN-γ – interferon gamma; TGF-β – transforming growth factor beta; TNF-α – tumor necrosis factor alpha; IL-6 – interleukin 6; IL-10 – interleukin 10; IL-18 – interleukin 18; iNOS – inducible nitric oxide; CD4 – cluster of differentiation 4; CD8 – cluster of differentiation 8; B cells – bursa-derived cells; NK cells – natural killer cells; Tregs – regulatory T cells; COX-2 – cyclooxygenase 2.

cancers. AgNPs derived from aqueous PGLE significantly reduced the viability of human cervical cancer cells (HeLa), showcasing a dose-dependent anti-cancer effect (Sarkar & Kotteeswaran, 2018). Aqueous PGLE pre-treatment in mice significantly bolstered antioxidant defenses and reduced genomic damage in bone marrow cells, indicating a protective role against chemotherapy-induced toxicity (Dassprakash, Arun, Abraham, & Premkumar, 2012). Methanol extracts from pomegranate leaves induced cell cycle arrest, inhibited cell proliferation, and triggered apoptosis in human multiple myeloma cells (U266), highlighting a potential chemopreventive effect (Kiraz et al., 2016). In prostate cancer studies, aqueous PGLE exhibited anti-proliferative effects against prostate cancer cells (LNCaP), with the ethyl acetate fraction showing pronounced efficacy in inhibiting the proliferation of PC3 prostate cancer cells (Elfiky, 2018; Orgil et al., 2014). PGLE has also demonstrated properties that affect the proliferation, apoptosis, and metastasis of various prostate cancer cell lines (Deng et al., 2018).

The anti-cancer properties of PGLE, spanning from liver to prostate cancer, underscore its potential as a multi-faceted natural agent for cancer treatment. The ability of PGLE to target various cancer cell lines through different mechanisms, including apoptosis induction, cell cycle arrest, anti-oxidant, anti-inflammatory and anti-proliferative effects (Fig. 4), positions it as a promising candidate for further development into therapeutic agents. The novel applications of PGLE in cancer prevention and treatment offer a glimpse into the future of oncology, where natural compounds play a pivotal role in combating malignancies.

3.4. Glycolipid metabolism of pomegranate leaves in managing diabetes, obesity, and associated complications

The exploration of pomegranate leaf extract (PGLE) in the regulation of glycolipid metabolism presents a groundbreaking approach to combat diabetes, obesity, and their related complications. This subsection highlights the innovative mechanisms and potential applications of PGLE in these metabolic disorders (Fig. 5).

3.4.1. Anti-hyperglycemic activity against diabetes

Diabetes mellitus, a growing global health concern, has shown a promising response to PGLE treatments. The ethanolic extract from the Nana variety of PGLE demonstrated a remarkable reduction in serum glucose, insulin, and HbA1c levels, showcasing a significant anti-diabetic effect *in vivo*, comparable to the pharmaceutical gliclazide (Elfiky, 2018). Aqueous PGLE outperformed hydroalcoholic extracts in α-amylase inhibition, suggesting superior anti-diabetic efficacy (Mohammed et al., 2020). Moreover, methanolic PGLE normalized critical blood and organ markers, indicating its potential to mitigate oxidative stress and restore metabolic balance in diabetic models (Pottathil et al., 2020). Notably, PGLE treatment preserved pancreatic architecture, suggesting a protective effect against ROS-induced damage (Pottathil et al., 2020).

3.4.2. Enzymatic inhibition and glycemic control

PGLE's ability to inhibit key enzymes, α-glucosidase and α-amylase, which are instrumental in polysaccharide digestion, positions it as a strategic agent to control postprandial blood glucose levels. The ethyl acetate fraction of PGLE demonstrated a multifaceted mechanism, enhancing glycogenesis, increasing glucose uptake by muscles, and reducing intestinal glucose absorption, independent of pancreatic insulin release (Patel, Bandawane, & Mhetre, 2014). Silver nanoparticles (AgNPs) synthesized with aqueous PGLE also showed potent enzyme inhibitory activity, highlighting a novel application in diabetes management (Saratale et al., 2018).

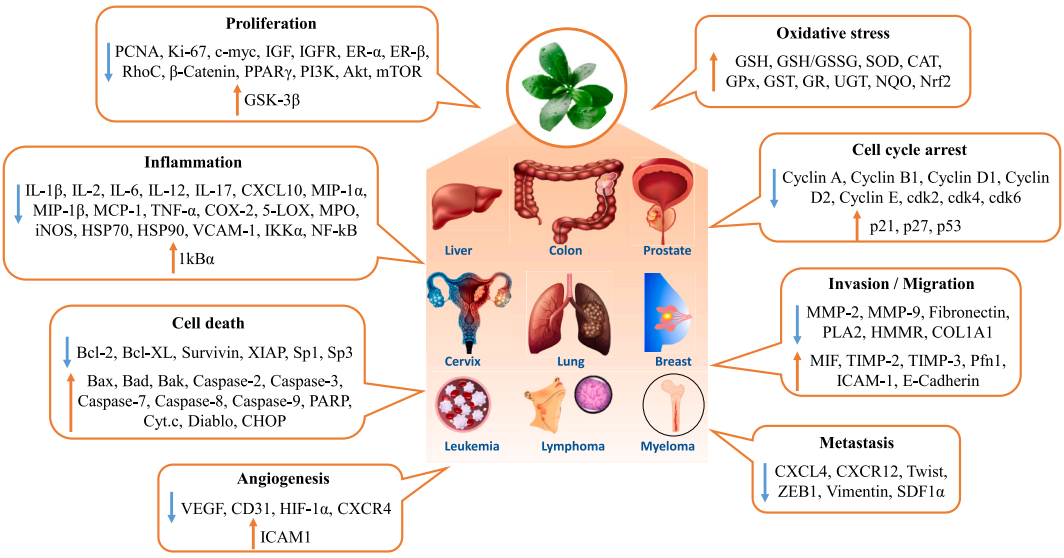

Fig. 4. Potential anticancer mechanisms and molecular targets of pomegranate leaves. Abbreviations: PCNA – proliferating cell nuclear antigen; Ki-67 – a marker for proliferation; c-myc – cellular myelocytomatosis oncogene; IGF – insulin-like growth factor; IGFR – insulin growth factor receptor; ER-α – estrogen receptor alpha; ER-β – estrogen receptor beta; RhoC – one isoform of Rho family subfamily; β-Catenin – beta-catenin/catenin beta-1; PPARγ – peroxisome proliferator-activated receptor gamma; PI3K – phosphoinositide 3-kinase; Akt – protein kinase B; mTOR – mammalian target of rapamycin; GSK-3β – glycogen synthase kinase-3 beta; IL-1β – interleukin-1 beta; IL-2 – interleukin 2; IL-6 – interleukin 6; IL-12 – interleukin 12; IL-17 – interleukin 17; CXCL10 – C-X-C motif chemokine 10; MIP-1α – macrophage inflammatory protein-1 alpha; MIP-1β – macrophage inflammatory protein-1 beta; MCP-1 – monocyte chemotactic protein-1; TNF-α – tumor necrosis factor alpha; COX-2 – cyclooxygenase 2; 5-LOX – 5-lipoxygenase; MPO – myeloperoxidase; iNOS – inducible nitric oxide; HSP70 –heat shock protein 70; HSP90 – heat shock protein; VCAM-1 – vascular cell adhesion molecule 1; IKKα – IκB kinase α; NF-kB – nuclear transcription factor kappa B; 1kBα – inhibitor of NF-kB; Bcl-2 – B-cell lymphoma 2; Bcl-XL – B-cell lymphoma-extra-large; XIAP – X-linked inhibitor of apoptosis protein; Sp1 – specificity protein 1; Sp3 – specificity protein 3; Bax – Bcl-2 associated X protein; Bad – Bcl-associated death promoter; PARP – poly (ADP-ribose) polymerase; Cyt.c – cytochrome *c*; Diablo, CHOP – cyclophosphamide, doxorubicin (Adriamycin) hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone; VEGF – vascular endothelial growth factor; CD31 – cluster of differentiation 31; HIF-1α – hypoxia-inducible factor 1-alpha; CXCR4 – C-X-C chemokine receptor type 4; ICAM1 – intercellular adhesion molecule 1; GSH – glutathione; GSSG – glutathione disulfide; SOD – superoxide dismutase; CAT – catalase; GPx – glutathione peroxidase; GST – glutathione S-transferase; GR – glutathione reductase; UGT – uridine 5'-diphospho-glucuronosyltransferase; NQO – 4-nitroquinoline 1-oxide; Nrf2 – nuclear factor erythroid 2-related factor 2; cdk2 – cyclin-dependent kinase 2; cdk4 – cyclin-dependent kinase 4; cdk6 – cyclin-dependent kinase 6; P21 – cyclin-dependent kinase inhibitor 1 A; p27 – cyclin-dependent kinase inhibitor 1B; p53 – tumor protein; MMP-2 – matrix metallopeptidase 2; MMP-9 – matrix metallopeptidase 9; PLA2 – phospholipase A2; HMMR – hyaluronan mediated motility receptor; COL1A1 – collagen type I alpha 1; MIF – macrophage migration inhibitory factor; TIMP-2 – tissue inhibitor of metalloproteinase 2; TIMP-3 – tissue inhibitor of metalloproteinase 3; Pfn1 – human profilin 1; CXCL4 – C-X-C motif chemokine; CXCR12 – C-X-C motif chemokine; Twist - Twist Transcription Factor; ZEB1 – zinc finger *E*-box binding homeobox 1; SDF1α – human stromal cell derived factor 1 alpha.

3.4.3. Prevention of Advanced Glycation End Products (AGEs)

AGEs are pivotal in the progression of diabetic complications. Methanolic PGLE has been identified as an effective AGE inhibitor, reducing oxidative protein damage and maintaining protein thiol groups (S. N. Mestry et al., 2018). This anti-glycation effect, coupled with the reduction of fasting blood glucose and AGEs, underscores PGLE's role in delaying diabetic complications (S. N. Mestry & Juvekar, 2017). The extract's efficacy in inhibiting AGE formation has been corroborated by comparative studies with other medicinal plants, with PGLE showing exceptional activity (Kaewnarin et al., 2014).

3.4.4. Impact on lipid metabolism and obesity

Hyperlipidemia, often associated with obesity, has been effectively managed with PGLE. Ethanolic PGLE improved serum lipid profiles and reduced atherogenic risk in the alloxan-induced diabetic models (Das & Barman, 2012). The extract's inhibition of acetylcholinesterase and butyrylcholinesterase suggests additional therapeutic applications (Bekir et al., 2013). Furthermore, PGLE's active components, including ellagic and gallic acids, have been found to attenuate lipid absorption and modulate lipase activity, offering a natural solution to managing hyperlipidemia (X. Yu et al., 2017).

3.4.5. Anti-cataract and anti-osteoporotic effects

The complications of diabetes extend to cataracts and osteoporosis, where PGLE has shown potential as a therapeutic agent. Its AR inhibitory and antioxidant properties suggest a protective effect against glucose-induced cataractogenesis (S. N. Mestry & Juvekar, 2017). Additionally, ethanolic PGLE demonstrated anti-osteoporotic activity in ovariectomized models, improving bone health markers in a dose-dependent manner (Halekunche, Burdipad, Kuppusamy, & Janadri, 2016).

The multifaceted effects of PGLE on glycolipid metabolism, from enzymatic inhibition to lipid profile modulation, present a novel and holistic approach to managing complex metabolic disorders. The potential of PGLE to serve as a natural, multi-targeted therapeutic agent is clear, with implications for dietary supplements and pharmaceutical development. Further research and clinical trials are warranted to fully harness the therapeutic potential of PGLE in the prevention and treatment of diabetes, obesity, and their complications, paving the way for innovative health solutions.

3.5. Neuroprotective effects of pomegranate leaves in neurological disorders

Pomegranate leaf extract (PGLE) has emerged as a promising natural remedy with neuroprotective properties, exhibiting significant potential in mitigating neurological disorders. The novelty of PGLE lies in its multi-targeted approach, impacting various pathways involved in neuroprotection and offering applications ranging from dietary supplements to therapeutic agents.

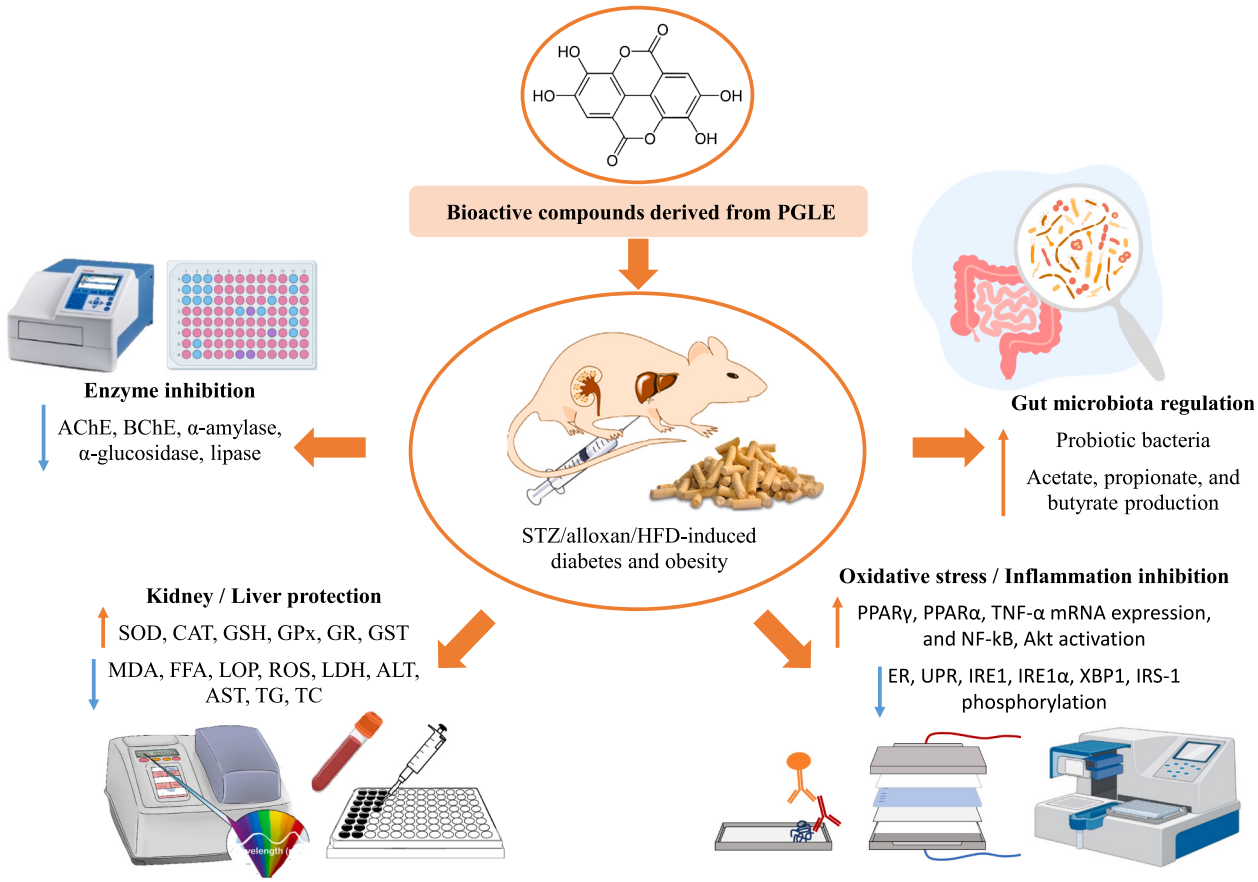

Fig. 5. Potential mechanisms of pomegranate leaves for regulating the glycolipid metabolism. Abbreviations: AChE – acetylcholinesterase; BChE – butyrylcholinesterase; SOD – superoxide dismutase; CAT – catalase; GSH – glutathione; GPx – glutathione peroxidase; GR – glutathione reductase; GST – glutathione S-transferase; MDA – malondialdehyde; FFA – free fatty acids; LOP – lipid peroxidation; ROS – reactive oxygen species; LDH – lactate dehydrogenase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; TG – triglyceride; TC – total cholesterol; PPARγ – peroxisome proliferator-activated receptor gamma; PPARα – peroxisome proliferator-activated receptor alpha; TNF-α – tumor necrosis factor alpha; NF-kB – nuclear transcription factor kappa B; Akt – protein kinase B; ER – endoplasmic reticulum; UPR – unfolded protein response; IRE1 – inositol-requiring enzyme 1; IRE1α – inositol-requiring enzyme 1α; XBP1 – X-box binding protein 1; IRS-1 – insulin receptor substrate 1.

3.5.1. *Anti-convulsant effect*

Research has demonstrated the potent anti-convulsant activity of ethanolic PGLE. In animal models, such as the maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizure models, PGLE effectively mitigated seizure severity. It abolished the hind limb extensor phase in the MES model and reduced the duration and frequency of seizures in a dose-dependent manner in the PTZ model (Das & Sarma, 2014). Methanolic PGLE further reinforced these findings, showcasing a dose-dependent alleviation of 6-Hz-induced seizures, outperforming other solvent extracts (L. Viswanatha, Venkataranganna, Prasad, & Ashok, 2016). These findings suggest PGLE's potential as an adjunct or alternative therapy in seizure management.

3.5.2. *Anxiolytic effect*

PGLE has also been recognized for its anxiolytic effects. Oral administration of PGLE was found to significantly increase brain gamma-aminobutyric acid (GABA) levels, which play a crucial role in regulating neuronal excitability and anxiety (L. Viswanatha et al., 2016). Behavioral studies using the elevated plus maze and mirror chamber methods revealed that ethanolic PGLE increased exploratory behavior and reduced anxiety-related parameters in mice (Das & Sarma, 2014). These anxiolytic properties highlight PGLE's potential as a natural anxiolytic agent.

3.5.3. *Neurotoxicity prevention*

The neuroprotective capabilities of PGLE extend to the prevention of neurotoxicity. Methanolic PGLE conferred substantial protection against global ischemia and reperfusion-induced deficits in neurological, motor, and cognitive functions in Wistar rats (Viswanatha et al., 2019). Additionally, PGLE extracts have been shown to attenuate amyloid β (Aβ) peptide-induced neurotoxicity in *Drosophila melanogaster*, a model organism for studying Alzheimer's disease. These extracts prolonged lifespan, improved locomotor abilities, and rescued neurodegeneration, with effects comparable to the standard drug donepezil (Ramasamy, Periyanayagam, & Sugithra, 2021). The presence of ursolic acid in PGLE is linked to these neuroprotective outcomes.

The therapeutic potential of PGLE in neurological disorders opens avenues for its incorporation into neuroprotective treatment regimens. Its ability to modulate GABAergic activity and prevent neurotoxicity positions it as a candidate for developing natural supplements aimed at supporting brain health. Moreover, the anti-convulsant and anxiolytic effects of PGLE could be harnessed in the formulation of novel therapeutics for epilepsy and anxiety disorders. The research underscores the need for further clinical trials to validate these applications and explore the full therapeutic potential of PGLE in neurology. In summary, the neuroprotective effects of PGLE, supported by preclinical studies, present a compelling case for its potential use in preventing and managing neurological disorders, with a promise for innovative health solutions derived from natural sources.

3.6. Anti-microbial properties of pomegranate leaves in combating infectious diseases

Pomegranate leaf extract (PGLE) is a potent anti-microbial agent, exhibiting a wide range of activities against bacteria, fungi, viruses, and parasites.

3.6.1. Anti-bacterial and anti-fungal activities

PGLE has been identified as a strong anti-bacterial agent, particularly effective against Gram-positive bacteria. Acetone extracts of PGLE have shown the highest anti-bacterial activity, surpassing other solvent extracts (Kaneria & Chanda, 2013). Methanolic extracts, when used in liquid soap formulations, retain both anti-bacterial and antioxidant properties (WMANK & Perera, 2016). These extracts have demonstrated efficacy against *Listeria monocytogenes* and *Staphylococcus aureus* with minimum inhibitory concentrations that are competitive with other plant extracts (Pallavali et al., 2019). PGLE's anti-fungal properties are notable, especially in inhibiting aflatoxin production by *Aspergillus parasiticus* in animal feed (Hassan, Sultana, Iqbal, Naz, & Abbas, 2017), and in combating *Cryptococcus gattii*, a significant fungal pathogen in immunocompromised individuals (Villis et al., 2021).

3.6.2. Anti-viral activity

PGLE's anti-viral capabilities have been increasingly recognized. Studies have shown its effectiveness in boosting immune responses against lymphocystis disease virus (LDV) in fish (Harikrishnan et al., 2010) and inhibiting HIV-1 integrase and reverse transcriptase activities, suggesting potential for HIV treatment development (Acquadro et al., 2017; Sanna et al., 2021). Additionally, PGLE and its constituent ellagic acid have shown promising results against Zika virus, indicating potential for preventive and therapeutic applications (Acquadro et al., 2020).

3.6.3. Anti-parasitic effects

In the realm of parasitology, PGLE has shown potential against *Haemonchus contortus*, a nematode affecting ruminants (Fikri, Ab, Zakaria, & Fariz, 2018), and has displayed larvicidal and repellent properties against mosquito vectors of malaria and Zika (Baranitharan et al., 2019). Furthermore, its efficacy in inhibiting β-haematin formation suggests anti-malarial activity (Akkawi et al., 2019), and its use in managing trypanosomosis has been demonstrated in animal models (Inabo & Fathuddin, 2011).

In fact, the antimicrobial activity of pomegranate extracts is multifaceted (Celiksoy & Heard, 2021), involving membrane disruption, enzyme inhibition, alteration in membrane fluidity and permeability, biofilm inhibition, quorum sensing interference, and specific chemical interactions dictated by the structure of polyphenolic compounds (Fig. 6). The complexity of these extracts and the interplay of different compounds also suggest a synergistic mechanism behind their antimicrobial effects.

Beyond its direct anti-microbial effects, PGLE has been explored for its utility in public health applications. Its anti-lice and anti-dandruff properties suggest potential for use in personal care products (Bhinge et al., 2021). The broad-spectrum activity of PGLE also positions it as a candidate for developing novel anti-microbial agents for use in healthcare settings, food preservation, and as a natural preservative in pharmaceuticals. Therefore, the extensive anti-microbial profile of PGLE, supported by empirical research, highlights its potential as a versatile and natural solution for managing a variety of infectious diseases and for application in health-related industries.

4. Current research and prospects

4.1. Recent findings

The significant growth in research related to pomegranate leaf over the recent ten years is shown in Fig. 7. The total number of papers published has increased steadily from 10 in 2014 to 95 in 2023, reflecting a rising interest in this field. Specifically, studies focused on bioactive compounds have shown a substantial increase, indicating a growing recognition of the diverse beneficial compounds found in pomegranate leaves. Research on bioactive activities has also expanded, suggesting that the potential health benefits of these compounds are being actively explored. The number of *in vitro* studies has risen consistently, highlighting the foundational work being conducted to understand the mechanisms of action at a chemical or cellular level. Similarly, *in vivo* studies have increased, demonstrating efforts to validate the therapeutic effects of pomegranate leaf extracts in animal models. This overall trend underscores the burgeoning scientific interest and ongoing efforts to elucidate the health benefits and applications of

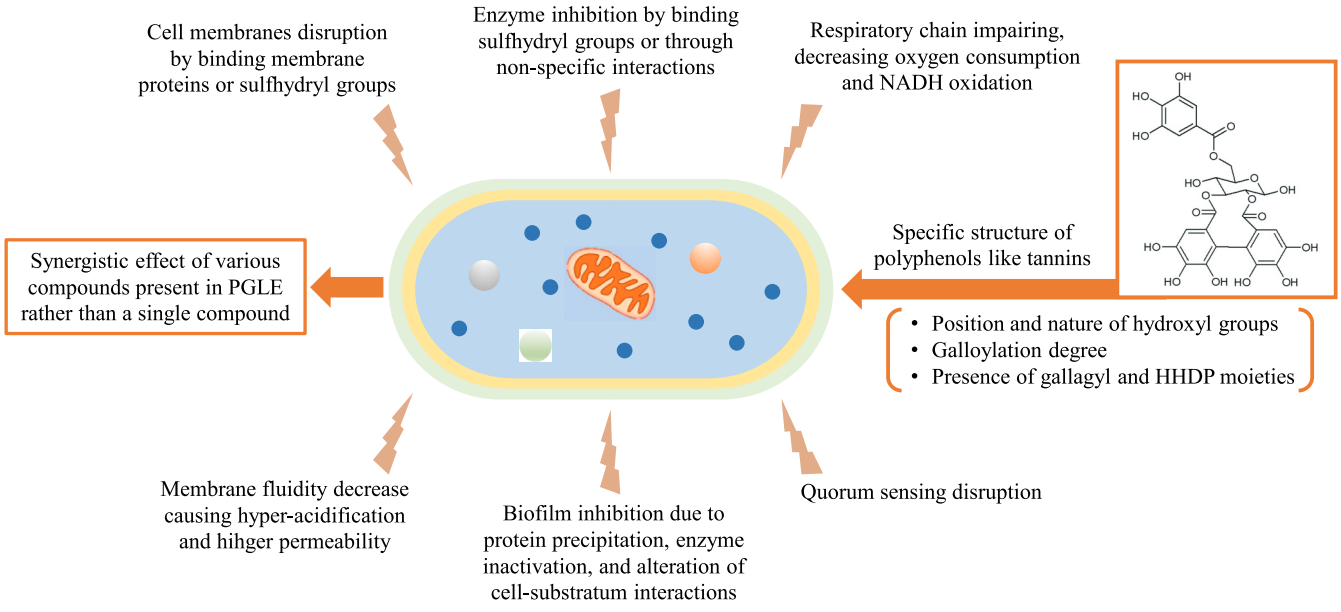

Fig. 6. Summarized mechanisms of anti-microbial effect of pomegranate leaves. Abbreviations: PGLE – pomegranate leaf extracts; NADH – β-nicotinamide adenine dinucleotide; HHDP – hexahydroxydiphenic acid.

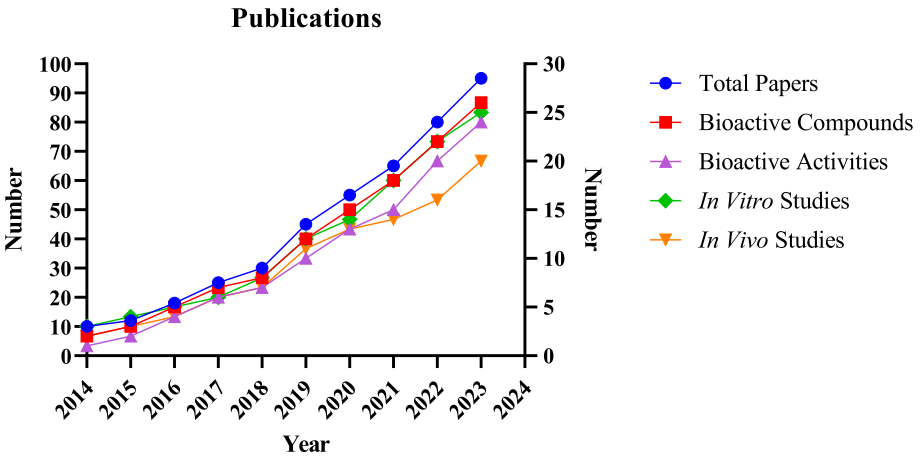

Fig. 7. Growth in research related to pomegranate leaf over the past decade using Scopus database. The total papers correspond to the left Y-axis. The number of articles related to bioactive compounds, bioactive activities, *in vitro* studies, and *in vivo* studies corresponds to the right Y-axis.

pomegranate leaf in various fields.

Pomegranate leaves (PGL) have garnered significant attention for their diverse biochemical properties and health benefits (Table S2). Recent studies highlight their antioxidant capabilities, which contribute to reducing oxidative stress in various body systems (Haseeb et al., 2017; S. Mestry et al., 2020; S. N. Mestry et al., 2018). Anti-inflammatory properties have been observed, particularly in reducing inflammation-related markers, offering potential therapeutic uses in inflammatory diseases (Gheith & El-Mahmoudy, 2017). Their anti-diabetic effects are notable, with research showing their potential in regulating blood glucose levels (Marques et al., 2016; Pinheiro et al., 2018; Pinheiro et al., 2019; Toda et al., 2020). PGL also exhibits strong anti-microbial properties, effective against a range of microbial infections (Bhinge et al., 2021; Sanna et al., 2021; Villis et al., 2021). Additionally, their anti-cancer potential has been a groundbreaking discovery, particularly in liver cancer studies, showing promising results in inhibiting cancer cell proliferation (Kumar et al., 2021; Rao & Krishnamurthy, 2019). Moreover, innovative applications of PGL extracts, such as the creation of silver nanoparticles (AgNPs) with enhanced DPPH scavenging capacity, have been explored by Saratale et al. (2018). This indicates the potential of PGL in nanotechnology and its application in health-related fields. These findings underline the vast potential of PGL in both food and pharmaceutical industries, warranting further research and development.

4.2. Pharmacokinetics and bioavailability of pomegranate leaves (PGL)

Recent research has begun to unravel the pharmacokinetics and bioavailability of compounds in PGL, although little has been reported. Fellah et al. (2020) focused on the phenolic composition of PGL, tracing the fate of these polyphenols during *in vitro* large intestine fermentation by using untargeted metabolomics. They discovered the catabolic pathway of phenolic metabolites and significant transformation in polyphenols, with flavonoids being the most prevalent class post-fermentation. This study provides insights into how PGL polyphenols might be metabolized in the human gut. Lei et al. (2003) explored the pharmacokinetics of ellagic acid, a key component in PGL. Their study in male Wistar rats showed that the max concentration of ellagic acid in plasma was 213 ng/mL only 0.55 h after oral administration of PGLE at 0.8 g/kg. Ellagic acid is rapidly absorbed and eliminated, suggesting poor absorption in the gastrointestinal tract. This research is pivotal in understanding how PGL compounds are processed in the body.

Additionally, Lan et al. (2009) investigated the transport of ellagic acid into HepG2 cells. They found a correlation with changes in total cholesterol in the cells, indicating the potential impact of PGL on lipid metabolism. Furthermore, research by Xueli et al. (2005) on brevifolin, another major ellagitannin in PGL, revealed similar pharmacokinetic properties to ellagic acid. This suggests that compounds with similar structures in PGL might exhibit comparable pharmacokinetic behaviors, aiding in the prediction of the overall pharmacokinetic profile of PGL tannins. These studies collectively advance our understanding of how PGL compounds behave in the body, crucial for developing effective therapeutic and nutritional applications.

4.3. Toxicity and safety of pomegranate leaves (PGL)

The use of various forms of PGL, including pastes, infusions, decoctions, and powders, has a long history of medicinal application. Generally, PGL is considered safe for human use. Various studies have assessed the toxicity of different PGL extracts in animals and microorganisms (Table S6).

In vitro studies have focused on the cytotoxic activity of PGL extracts against various cell lines, using the MTT assay. Elbatanony et al. (2019) evaluated the safety of lipoidal and natural pigment extracts of PGL on skin fibroblast normal cells (BJ1), hepatocellular carcinoma cells (HepG2), and breast cancer cells (MCF7), finding them to be safe at certain concentrations. The cytotoxic effects vary depending on the solvent used for extraction, as demonstrated by Bekir et al. (2013) and other studies assessing the impact on different cancer cell lines.

In vivo study, conducted by Das and Barman (2012), have utilized acute oral toxicity tests to evaluate the adverse effects of PGL extracts. These studies generally indicate the non-toxic nature of PGL at certain dosages in animal models. However, the extent of toxicity can vary based on the type of extract and the dosage used.

A systematic review (Zare et al., 2023) analyzed the adverse effects reported in clinical studies of pomegranate, including a total of 66 clinical articles. This review indicates that pomegranate and its extracts are generally safe, with few reported side effects, primarily gastrointestinal issues and allergic reactions. Nonetheless, the scarcity of clinical trials specifically focused on the therapeutic and toxicological aspects of PGL underscores the need for more robust controlled trials and thorough documentation of potential side effects.

On the other hand, while the extraction process using organic solvents like petroleum ether and methanol can isolate beneficial compounds, it is crucial to consider the potential health risks associated with their residues. Therefore, proper purification and removal of solvent residues are essential to ensure the safety of the extracts for human use.

4.4. Clinical trials and human studies

Indeed, recent clinical trials have highlighted the health benefits of pomegranate. The study by Baghdadi et al. (2023) reviewed the impact

of various pomegranate parts on cardiovascular disease risk factors in patients undergoing maintenance dialysis. This systematic review found significant anti-hypertensive, antioxidant, and anti-inflammatory effects, along with improved lipid profiles. In another significant study, Barghchi et al. (2023) investigated the influence of pomegranate peel extract on non-alcoholic fatty liver disease (NAFLD) patients. This trial revealed notable improvements in various metabolic syndrome risk factors and hepatic steatosis, although no significant changes were observed in insulin resistance metrics.

However, current research on pomegranate leaves (PGL) is predominantly in the preclinical stage, with most studies focusing on *in vitro* or *in vivo* experiments using extracts or fractions. This gap highlights a significant need for clinical trials to validate the therapeutic potential of PGL. While the phenolic composition of PGL, including compounds like ellagic acid and punicalagin, has been explored for pharmacological properties such as anti-inflammation, these have not yet been tested in clinical trials. To fully understand and utilize the health benefits of PGL, future research should prioritize randomized clinical trials with large sample sizes. Additionally, expanding the medicinal profile of PGL by-products and isolating more components for bioactivity assessment is recommended to optimize the health-promoting potential of PGL.

4.5. Innovations in application

Pomegranate leaves (PGL) are being innovatively applied across various industries due to their diverse biological properties. In the food industry, PGL is gaining popularity for products like pomegranate leaf tea and baked goods, praised for their health benefits and convenience (M. Yu et al., 2023). PGL blends with other botanicals have been proposed as novel food additives owing to their antioxidant potency and thermal stability (Mirza & Dabhade, 2018). Additionally, PGL shows promise as a natural fungicide, effectively combating fungal pathogens in post-harvest crops (Koka, Bhat, & Wani, 2020). In pharmacology, nutraceuticals, and cosmetics, PGL is used in medicinal supplements, soaps, shampoos, and anti-aging creams, demonstrating anti-dandruff, anti-lice, and hair growth-promoting effects (Bhinge et al., 2021). The presence of ellagic acid and its precursors like granatine and ellagitannin enhances its role in liver protection, cancer prevention, and as a blood coagulant, meeting a growing market demand (Acquadro et al., 2020). Environmentally, PGL-derived metal nanoparticles improve water quality by removing harmful dyes and toxins (Kamath, Chandra, & Jeppu, 2020; Vidovix, Quesada, Bergamasco, Vieira, & Vieira, 2021). In agriculture, PGL aids in preserving animal health by inhibiting feed degradation and combating parasites (Fathuddin & Inabo, 2017; Hassan et al., 2017). Manufacturing applications include using PGL as a natural colorant in paints and in petroleum distillate purification and corrosion inhibition (Abboud et al., 2016; Mohajer, Taha, & Azmi, 2016; Sadare & Daramola, 2019). This comprehensive utilization of PGL highlights its growing versatility and potential across various sectors, suggesting a promising future for PGL-based products and applications (Fig. S1).

4.6. Challenges and limitations

Recent research on pomegranate leaves (PGL) has revealed several challenges and limitations. One significant issue is compound stability, which affects the consistency and efficacy of PGL-based products. The variability in leaf composition due to different collection times and cultivation conditions poses another challenge, impacting the uniformity of bioactive compounds in PGL extracts (Zhang et al., 2010). This variability can influence both the therapeutic potential and safety profile of PGL-derived products. Furthermore, potential side effects, especially in the context of long-term use or high dosages, are not yet fully understood and require further investigation. Addressing these challenges is crucial for the development of reliable and safe PGL-based applications in various industries.

4.7. Future directions

The ongoing research on pomegranate leaves (PGL) should continue to evolve in several critical areas. Advanced analytical methods, including liquid chromatography and nuclear magnetic resonance, should be employed to isolate and understand the chemical structure of PGL's monomer components. Exploring the pharmacological activities of these components could lead to novel natural drugs for clinical use. There's a need for more in-depth clinical trials to validate PGL's therapeutic benefits, especially in preventing and treating cardiovascular diseases and hypertension. Utilizing emerging technologies such as metabolomics and genomics could uncover new bioactive compounds, enhancing PGL's medicinal profile. Understanding the specific mechanisms of action of PGL, particularly in anti-oxidation and blood sugar regulation, requires further investigation to determine whether these effects result from individual components or synergistic interactions. Another aspect involves examining the impact of processing methods on the bioactive substances in PGL and optimizing sustainable practices in its cultivation and processing. This will ensure efficacy while maximizing the utilization rate of PGL. Interestingly, considering the potential health risks of organic extraction solvents, complex of purification process, and cost of residual removal, it is really suggested to make PGL into different teas, like green tea or fermented tea, which is recognized as natural functional food. Finally, efforts should be made to transition from laboratory research to industrial production, promoting PGL's comprehensive application in food, medicine, and health products. With the deepening of research, PGL is poised to have a significant impact on various industries.

5. Conclusion

This review has comprehensively delineated the multifaceted potential of pomegranate leaf (PGL), positioning it as a treasure trove of bioactive compounds with vast therapeutic capabilities. The intricate interplay of phytochemicals like phenolic acids, tannins, flavonoids, and alkaloids in PGL presents promising avenues for treating a spectrum of health issues, from metabolic disorders and cancer to infectious diseases and neurological conditions. Our analysis, spanning two decades of *in vitro*, *in vivo*, and *in silico* studies, firmly establishes PGL's role in health promotion and disease mitigation.

However, a noticeable gap in clinical trials underscores an urgent need for research that translates these preliminary findings into human applications. Bridging this gap is essential for validating PGL's efficacy and safety in therapeutic contexts. The potential of PGL extends beyond healthcare, offering sustainable and innovative applications in the food, pharmaceutical, nutraceutical, and cosmetic industries. This exploration is just the beginning, with the journey ahead ripe with possibilities for uncovering the full spectrum of PGL's benefits. The future beckons with promises of new discoveries and applications, solidifying PGL's position as a pivotal element in advancing health science and environmental sustainability.

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CRediT authorship contribution statement

Manyou Yu: Writing – original draft, Visualization, Investigation. **Irene Gouvinhas:** Writing – original draft. **Jian Chen:** Methodology, Investigation. **Yongqing Zhu:** Methodology, Investigation. **Junlin Deng:** Validation, Investigation. **Zhuoya Xiang:** Validation,

Investigation. **Paula Oliveira:** Validation, Supervision. **Chen Xia:** Validation, Supervision, Funding acquisition. **Ana Barros:** Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101587.

References

Abboud, Y., Tanane, O., Bouari, A. E., Salghi, R., Hammouti, B., Chetouani, A., & Jodeh, S. (2016). Corrosion inhibition of carbon steel in hydrochloric acid solution using pomegranate leave extracts. *Corrosion Engineering, Science and Technology, 51*(8), 557–565. https://doi.org/10.1179/1743278215Y.0000000058

Acquadro, S., Civra, A., Cagliero, C., Marengo, A., Rittà, M., Francese, R., … Lembo, D. (2020). *Punica granatum* leaf ethanolic extract and ellagic acid as inhibitors of Zika virus infection. *Planta Medica, 86*(18), 1363–1374. https://doi.org/10.1055/a-1232-5705

Acquadro, S., Esposito, F., Sanna, C., Caredda, A., Marengo, A., Cagliero, C., … Rubiolo, P. (2017). Punica granatum leaves as a potential source of bioactive molecules. In *Paper presented at the international plant science conference–112*. Congresso della Società Botanica Italiana onlus.

Akkawi, M., Abu-Lafi, S., & Abu-Remeleh, Q. (2019). Phytochemical screening of pomegranate juice, peels, leaves and membranes water extracts and their effect on *β*-hematin formation, a comparative study. *Pharmacy & Pharmacology International Journal, 7*, 193–200. https://doi.org/10.15406/ppij.2019.07.00251

Al-Muammar, M. N., & Khan, F. (2012). Obesity: The preventive role of the pomegranate (*Punica granatum*). *Nutrition, 28*(6), 595–604. https://doi.org/10.1016/j.nut.2011.11.013

Amri, Z., Zaouay, F., Lazreg-Aref, H., Soltana, H., Mneri, A., Mars, M., & Hammami, M. (2017). Phytochemical content, fatty acids composition and antioxidant potential of different pomegranate parts: Comparison between edible and non edible varieties grown in Tunisia. *International Journal of Biological Macromolecules, 104*, 274–280. https://doi.org/10.1016/j.ijbiomac.2017.06.022

Angamuthu, D., Purushothaman, I., Kothandan, S., & Swaminathan, R. (2019). Antiviral study on *Punica granatum* L., *Momordica charantia* L., *Andrographis paniculata* Nees, and *Melia azedarach* L., to human herpes Virus-3. *European Journal of Integrative Medicine, 28*, 98–108. https://doi.org/10.1016/j.eujim.2019.04.008

Ankita, P., Deepti, B., & Nilam, M. (2015). Flavonoid rich fraction of *Punica granatum* improves early diabetic nephropathy by ameliorating proteinuria and disturbed glucose homeostasis in experimental animals. *Pharmaceutical Biology, 53*(1), 61–71.

Baghdadi, G., Shidfar, F., & Dehnad, A. (2023). The effect of pomegranate consumption on cardiovascular risk factors in hemodialysis patients: A systematic review of clinical trials. *Phytotherapy Research, 37*(11), 4963–4975. https://doi.org/10.1002/ptr.7961

Balamurugan, C., Karuppasamy, R., Sivaraj, C., Saraswathi, K., & Arumugam, P. (2021). Cytotoxic activity of leaves extract of pomegranate (*Punica granatum* L.). *Journal of Pharmacognosy and Phytochemistry, 10*(1), 1982–1985. https://doi.org/10.22271/phyto.2021.v10.i1ab.13640

Balwani, S., Nandi, D., Jaisankar, P., & Ghosh, B. (2011). 2-Methyl-pyran-4-one-3-O-β-d-glucopyranoside isolated from leaves of *Punica granatum* inhibits the TNFα-induced cell adhesion molecules expression by blocking nuclear transcription factor-κB (NF-κB). *Biochimie, 93*(5), 921–930. https://doi.org/10.1016/j.biochi.2011.01.010

Baranitharan, M., Tamizhazhagan, V., Kovendan, K., & Senthilmurugan, S. (2019). *Punica granatum*-based green ethanolic extract as highly effective and eco-friendly larvicide, repellent against medically important mosquito vectors. *Entomology and Applied Science Letters, 6*(2–2019), 33–41.

Barghchi, H., Milkarizi, N., Belyani, S., Norouzian Ostad, A., Askari, V. R., Rajabzadeh, F., … Nematy, M. (2023). Pomegranate (*Punica granatum* L.) peel extract ameliorates metabolic syndrome risk factors in patients with non-alcoholic fatty liver disease: A randomized double-blind clinical trial. *Nutrition Journal, 22*(1), 40. https://doi.org/10.1186/s12937-023-00869-2

Bekir, J., Mars, M., Souchard, J. P., & Bouajila, J. (2013). Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves. *Food and Chemical Toxicology, 55*, 470–475. https://doi.org/10.1016/j.fct.2013.01.036

Bhandary, S. K. N. S. K., Bhat, V. S. P. S. K., & Bekal, M. P. (2012). Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *Journal of Health and Allied Sciences NU, 02*(04), 34–38. https://doi.org/10.1055/s-0040-1703609

Bhinge, S. D., Bhutkar, M. A., Randive, D. S., Wadkar, G. H., Todkar, S. S., Savali, A. S., & Chittapurkar, H. R. (2021). Screening of hair growth promoting activity of *Punica granatum* L. (pomegranate) leaves extracts and its potential to exhibit antidandruff and anti-lice effect. *Heliyon, 7*(4), Article e06903. https://doi.org/10.1016/j.heliyon.2021.e06903

Cavalcanti, R. N., Navarro-Díaz, H. J., Santos, D. T., Rostagno, M. A., & Meireles, M. A. A. (2012). Supercritical carbon dioxide extraction of polyphenols from pomegranate (*Punica granatum* L.) leaves: Chemical composition, economic evaluation and chemometric approach. *Journal of. Food Research, 1*(3), 282.

Celiksoy, V., & Heard, C. M. (2021). Antimicrobial potential of pomegranate extracts. In *Pomegranate*. IntechOpen.

Das, S., & Barman, S. (2012). Antidiabetic and antihyperlipidemic effects of ethanolic extract of leaves of *Punica granatum* in alloxan-induced non-insulin-dependent diabetes mellitus albino rats. *Indian Journal Of Pharmacology, 44*(2), 219–224. https://doi.org/10.4103/0253-7613.93853

Das, S., & Sarma, P. (2014). A study on the anticonvulsant and antianxiety activity of ethanolic extract of *Punica granatum* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(2), 389–392.

Dassprakash, M. V., Arun, R., Abraham, S. K., & Premkumar, K. (2012). *In vitro* and *in vivo* evaluation of antioxidant and antigenotoxic potential of *Punica granatum* leaf extract. *Pharmaceutical Biology, 50*(12), 1523–1530. https://doi.org/10.3109/13880209.2012.689771

Deng, Y.-L., Li, Y.-L., Zheng, T.-T., Hu, M.-X., Ye, T.-H., Xie, Y.-M., & Yin, W.-Y. (2018). The extract from *Punica Granatum* (pomegranate) leaves promotes apoptosis and impairs metastasis in prostate cancer cells. *Sichuan da xue xue bao. Yi xue ban = Journal of Sichuan University. Medical science edition, 49*(1), 8–12. Retrieved from http://europepmc.org/abstract/MED/29737081.

Durgadevi, K., Marueen, J. A. A., Gowri, R., & Ramamurthy, V. (2018). A study of phytochemical screening and antibacterial activity of leaves extract of *Punica granatum*. *The Pharma Innovation Journal, 7*, 173–177.

Elbatanony, M. M., El-Feky, A. M., Hemdan, B. A., & Azab El-Liethy, M. (2019). Assessment of the antimicrobial activity of the lipoidal and pigment extracts of *Punica granatum* L. leaves. *Acta Ecologica Sinica, 39*(1), 89–94. https://doi.org/10.1016/j.chnaes.2018.05.003

Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., & Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plant Research, 6*(32), 4724–4730. https://doi.org/10.5897/JMPR11.995

Elfiky, A. M. A. (2018). *A pharmacognosical study of Punica granatum var. nana cultivated in Egypt*. CU Theses.

FAO. (2021). FAOSTAT statistical database. Food and agriculture Organization of the United Nations. Retrieved from https://www.fao.org/statistics/en. (Accessed 6 June 2024).

Farag, R. S., Abdel-Latif, M. S., Emam, S. S., & Tawfeek, L. S. (2014). Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. *Agricultural Soil Science, 1*(6), 86–93.

Fathuddin, M. M., & Inabo, H. I. (2017). In vitro Antitrypanosomal potential of chloroform leaf extract of *Punica granatum* L. on Trypanosoma brucei brucei and Trypanosoma evansi. *Microbiology Research, 8*(1), 24–27. Retrieved from https://www.mdpi.com/2036-7481/8/1/6963.

Fellah, B., Bannour, M., Rocchetti, G., Lucini, L., & Ferchichi, A. (2018). Phenolic profiling and antioxidant capacity in flowers, leaves and peels of Tunisian cultivars of *Punica granatum* L. *Journal of Food Science and Technology, 55*(9), 3606–3615. https://doi.org/10.1007/s13197-018-3286-8

Fellah, B., Rocchetti, G., Senizza, B., Giuberti, G., Bannour, M., Ferchichi, A., & Lucini, L. (2020). Untargeted metabolomics reveals changes in phenolic profile following *in vitro* large intestine fermentation of non-edible parts of *Punica granatum* L. *Food Research International, 128*, Article 108807. https://doi.org/10.1016/j.foodres.2019.108807

Fikri, S. F. F., Ab, N. A. I. I. S., Zakaria, R., & Fariz, S. (2018). *In vitro* anti-parasitic activities of pomegranate, *Punica granatum* against parasitic nematodes of ruminants. *Malaysian Hournal of Veterinary Research*, 1–9. ISSN 2180-3897. Journal Contribution.

Ge, S., Duo, L., Wang, J., GegenZhula, Yang, J., Li, Z., & Tu, Y. (2021). A unique understanding of traditional medicine of pomegranate, *Punica granatum* L. and its current research status. *Journal of Ethnopharmacology, 271*, Article 113877. https://doi.org/10.1016/j.jep.2021.113877

Gheith, I., & El-Mahmoudy, A. (2017). Potent antioxidant and anti-inflammatory potentials of *Punica granatum* leaf and flower hydromethanolic extracts *in vitro*. *Bioscience Journal, 33*(2). https://doi.org/10.14393/BJ-v33n2-33736

Haji, A., Shahmoradi Ghaheh, F., & Indrie, L. (2023). Pomegranate fallen leaves as a source of natural dye for mordant-free dyeing of wool. *Coloration Technology, 139*(2), 165–170. https://doi.org/10.1111/cote.12651

Halekunche, Y., Burdipad, G., Kuppusamy, S., & Janadri, S. (2016). Anti-osteoporotic activity of ethanol extract of leave on Punica granatum s ovariectomized rats. *Asian Journal of Pharmacy and Pharmacology, 2*(4), 85–92.

Harikrishnan, R., Heo, J., Balasundaram, C., Kim, M.-C., Kim, J.-S., Han, Y.-J., & Heo, M.-S. (2010). Effect of *Punica granatum* solvent extracts on immune system and disease resistance in *Paralichthys olivaceus* against lymphocystis disease virus (LDV). *Fish & Shellfish Immunology, 29*(4), 668–673. https://doi.org/10.1016/j.fsi.2010.07.006

Haseeb, A., Khan, N. M., Ashruf, O. S., & Haqqi, T. M. (2017). A polyphenol-rich pomegranate fruit extract suppresses NF-κB and IL-6 expression by blocking the activation of IKKβ and NIK in primary human chondrocytes. *Phytotherapy Research, 31*(5), 778–782. https://doi.org/10.1002/ptr.5799

Hassan, S. M., Sultana, B., Iqbal, M., Naz, S., & Abbas, M. (2017). Anti-aflatoxigenic activity of *Punica granatum* and *Ziziphus jujuba* leaves against Aspergillus parasiticus inoculated poultry feed: Effect of storage conditions. *Biocatalysis and Agricultural Biotechnology, 10*, 104–112. https://doi.org/10.1016/j.bcab.2017.02.003

Inabo, H., & Fathuddin, M. (2011). Antitrypanosomal potentials of ethanolic leaf extracts of *Punica granatum* against *Trypanosoma brucei brucei* infection. *Bayero Journal of Pure and Applied Sciences, 4*(2), 35–40. https://doi.org/10.4314/bajopas.v4i2.7

Jurenka, J. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alternative Medicine Review, 13*(2).

Kaewnarin, K., Niamsup, H., Shank, L., & Rakariyatham, N. (2014). Antioxidant and antiglycation activities of some edible and medicinal plants. *Chiang Mai Journal of Science, 41*(1), 105–116. http://cmuir.cmu.ac.th/jspui/handle/6653943832/53191.

Kamath, V., Chandra, P., & Jeppu, G. P. (2020). Comparative study of using five different leaf extracts in the green synthesis of iron oxide nanoparticles for removal of arsenic from water. *International Journal of Phytoremediation, 22*(12), 1278–1294. https://doi.org/10.1080/15226514.2020.1765139

Kaneria, M. J., Bapodara, M. B., & Chanda, S. V. (2012). Effect of extraction techniques and solvents on antioxidant activity of pomegranate (*Punica granatum* L.) leaf and stem. *Food Analytical Methods, 5*(3), 396–404. https://doi.org/10.1007/s12161-011-9257-6

Kaneria, M. J., & Chanda, S. V. (2013). The effect of sequential fractionation technique on the various efficacies of pomegranate (*Punica granatum* L.). *Food Analytical Methods, 6*(1), 164–175. https://doi.org/10.1007/s12161-012-9412-8

Kasai, K., Yoshimura, M., Koga, T., Arii, M., & Kawasaki, S. (2006). Effects of oral administration of ellagic acid-rich pomegranate extract on ultraviolet-induced pigmentation in the human skin. *Journal of Nutritional Science and Vitaminology, 52*(5), 383–388. https://doi.org/10.3177/jnsv.52.383

Kiraz, Y., Neergheen-Bhujun, V. S., Rummun, N., & Baran, Y. (2016). Apoptotic effects of non-edible parts of *Punica granatum* on human multiple myeloma cells. *Tumor Biology, 37*(2), 1803–1815. https://doi.org/10.1007/s13277-015-3962-5

Koka, J. A., Bhat, M. Y., & Wani, A. H. (2020). Allelopathic effect of leaf extracts of *Punica granatum* and *Spiraea prunifolia* against post-harvest rot of tomato and brinjal. *Journal of Drug Delivery and Therapeutics, 10*(2–s), 1–6. https://doi.org/10.22270/jddt.v10i2-s.3948

Kolar, F. R., Lingasur, S. M., Kumathalli, T. M., & Gurikar, S. A. (2021). Comparative analysis of phytochemical constituents and antioxidant activity of two pomegranate (*Punica granatum* L.) cultivars. *Israel Journal of Plant Sciences, 1*(aop), 1–10.

Kumar, M., Ranjan, R., Kumar, A., Sinha, M. P., Srivastava, R., Subarna, S., & Kumar Mandal, S. (2021). Hepatoprotective activity of silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* against induced hepatotoxicity in rats. *Nova Biologica Reperta, 7*(4), 381–389.

Lan, J., Lei, F., Hua, L., Wang, Y., Xing, D., & Du, L. (2009). Transport behavior of ellagic acid of pomegranate leaf tannins and its correlation with total cholesterol alteration in HepG2 cells. *Biomedical Chromatography, 23*(5), 531–536. https://doi.org/10.1002/bmc.1150

Lei, F., Xing, D.-M., Xiang, L., Zhao, Y.-N., Wang, W., Zhang, L.-J., & Du, L.-J. (2003). Pharmacokinetic study of ellagic acid in rat after oral administration of pomegranate leaf extract. *Journal of Chromatography B, 796*(1), 189–194. https://doi.org/10.1016/S1570-0232(03)00610-X

Li, Y., Yang, F., Zheng, W., Hu, M., Wang, J., Ma, S., … Yin, W. (2016). *Punica granatum* (pomegranate) leaves extract induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in non-small cell lung cancer *in vitro*. *Biomedicine & Pharmacotherapy, 80*, 227–235. https://doi.org/10.1016/j.biopha.2016.03.023

Marques, L. C., Pinheiro, A. J., Araújo, J. G., de Oliveira, R. A., Silva, S. N., Abreu, I. C., … Silbiger, V. N. (2016). Anti-inflammatory effects of a pomegranate leaf extract in LPS-induced peritonitis. *Planta Medica, 82*(17), 1463–1467. https://doi.org/10.1055/s-0042-108856

Melgarejo-Sánchez, P., Núñez-Gómez, D., Martínez-Nicolás, J. J., Hernández, F., Legua, P., & Melgarejo, P. (2021). Pomegranate variety and pomegranate plant part, relevance from bioactive point of view: A review. *Bioresources and Bioprocessing, 8*(1), 2. https://doi.org/10.1186/s40643-020-00351-5

Mestry, S., Gawali, N., Pai, S., Gursahani, M., Dhodi, J., Munshi, R., & Juvekar, A. (2020). *Punica granatum* improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. *Journal of Ayurveda and integrative medicine, 11*(1), 16–23. https://doi.org/10.1016/j.jaim.2017.09.006

Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum* Linn. leaves extract. *Journal of Traditional and Complementary Medicine, 7*(3), 273–280. https://doi.org/10.1016/j.jtcme.2016.06.008

Mestry, S. N., Gawali, N. B., Gursahani, M. S., Pai, S. A., Dhodi, J. B., & Juvekar, A. R. (2018). Inhibition of advanced glycation end products by *Punica granatum* Linn. leaves and its antioxidant activity. *Oriental Pharmacy and Experimental Medicine, 18*(2), 97–105. https://doi.org/10.1007/s13596-018-0309-y

Mestry, S. N., & Juvekar, A. R. (2017). Aldose reductase inhibitory potential and anti-cataract activity of *Punica granatum* Linn. leaves against glucose-induced cataractogenesis in goat eye lens. *Oriental Pharmacy and Experimental Medicine, 17*(3), 277–284. https://doi.org/10.1007/s13596-017-0274-x

Mirza, S. R., & Dabhade, S. B. (2018). Identification of novel food additive antioxidant from *Psidium guajava, Moringa oliefera, Punica granatum* blend leaves extracts. *International Research Journal of Engineering and Technology, 5*(5), 1293–1305.

Mohajer, S., Taha, R. M., & Azmi, S. Z. (2016). Phytochemical screening and potential of natural dye colourant from pomegranate (*Punica granatum* L.). *Pigment & Resin Technology, 45*(1), 38–44. https://doi.org/10.1108/PRT-10-2014-0100

Mohammed, C., Mohamed, A., Ahmed, A., & Mariod, A. (2020). Antioxidant and anti-diabetic activity of pomegranate (*Punica granatum* L.) leaves extracts. *Foods and Raw materials, 8*(2), 329–336.

Negi, P. S., Jayaprakasha, G. K., & Jena, B. S. (2003). Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry, 80*(3), 393–397. https://doi.org/10.1016/S0308-8146(02)00279-0

Ono, N. N., Bandaranayake, P. C. G., & Tian, L. (2012). Establishment of pomegranate (*Punica granatum*) hairy root cultures for genetic interrogation of the hydrolyzable tannin biosynthetic pathway. *Planta, 236*(3), 931–941. https://doi.org/10.1007/s00425-012-1706-y

Orgil, O., Schwartz, E., Baruch, L., Matityahu, I., Mahajna, J., & Amir, R. (2014). The antioxidative and anti-proliferative potential of non-edible organs of the pomegranate fruit and tree. *LWT - Food Science and Technology, 58*(2), 571–577. https://doi.org/10.1016/j.lwt.2014.03.030

Ouédraogo, S., Traoré, T. K., Ouedraogo, B., Atchadé, B. C., Coulibaly, A., Lompo, M., … Semdé, R. (2021). Phytochemical characteristics and pharmacological properties studies of the powders of *Punica granatum* L. (Lythraceae) leaves from seven regions of Burkina Faso with a view to standardizing. *Journal of Advances in Medical and Pharmaceutical Sciences, 23*(1), 1–11. Retrieved from https://www.scilit.net/article/7626a8c1f4d5de5bf2d68746f69b92d4.

Pallavali, R. R., Avula, S., Degati, V. L., Penubala, M., Damu, A. G., & Durbaka, V. R. P. (2019). Data of antibacterial activity of plant leaves crude extract on bacterial isolates of wound infections. *Data in Brief, 24*, Article 103896. https://doi.org/10.1016/j.dib.2019.103896

Patel, A. N., Bandawane, D. D., & Mhetre, N. K. (2014). Pomegranate (*Punica granatum* Linn.) leaves attenuate disturbed glucose homeostasis and hyperglycemia mediated hyperlipidemia and oxidative stress in streptozotocin induced diabetic rats. *European Journal of Integrative Medicine, 6*(3), 307–321. https://doi.org/10.1016/j.eujim.2014.03.009

Pinheiro, A. J. M. C. R., Gonçalves, J. S., Dourado, Á. W. A., de Sousa, E. M., Brito, N. M., Silva, L. K., … Lima-Neto, L. G. (2018). *Punica granatum* L. leaf extract attenuates lung inflammation in mice with acute lung injury. *Journal of Immunology Research, 2018*, Article 6879183. https://doi.org/10.1155/2018/6879183

Pinheiro, A. J. M. C. R., Mendes, A. R. S., Neves, M. D. F.d. J., Prado, C. M., Bittencourt-Mernak, M. I., Santana, F. P. R., … Lima-Neto, L. G. (2019). Galloyl-hexahydroxydiphenoyl (HHDP)-glucose isolated from *Punica granatum* L. leaves protects against lipopolysaccharide (LPS)-induced acute lung injury in BALB/c mice. *Frontiers in Immunology, 10*(1978). https://doi.org/10.3389/fimmu.2019.01978

Pottathil, S., Nain, P., Morsy, M. A., Kaur, J., Al-Dhubiab, B. E., Jaiswal, S., & Nair, A. B. (2020). Mechanisms of antidiabetic activity of methanolic extract of *Punica granatum* leaves in nicotinamide/streptozotocin-induced type 2 diabetes in rats. *Plants, 9*(11). https://doi.org/10.3390/plants9111609

Ramamurthy, V., & Marueen, J. A. A. (2018). A study of phytochemical screening and antioxidant activity of ethanolic leaves extract of *Punica granatum. International Journal of Research and Analytical Reviews, 5*(3), 794–800.

Ramasamy, N., Periyanayagam, K., & Sugithra, B. (2021). Pomegranate (*Punica granatum*. Linn. var. Ganesh) leaf extracts ameliorates neurotoxicity in transgenic *Drosophila* expressing human amyloid pathology. *Indian Journal of Pharmaceutical and Biological Research, 9*(01), 1–7. Available from: http://ijpbr.in/index.php/IJPBR/article/view/1000.

Ranjha, M. M. A. N., Shafique, B., Wang, L., Irfan, S., Safdar, M. N., Murtaza, M. A., … Nadeem, H. R. (2021). A comprehensive review on phytochemistry, bioactivity and medicinal value of bioactive compounds of pomegranate (*Punica granatum*). *Advances in Traditional Medicine*. https://doi.org/10.1007/s13596-021-00566-7

Rao, S. P., & Krishnamurthy, V. (2019). Ethanol extract of *Punica granatum* L. leaf in combination with gallic acid ameliorates liver dysfunction in high-fat diet-induced obese rats. *Indian Journal of Pharmaceutical Sciences, 81*(4), 673–680. https://doi.org/10.36468/pharmaceutical-sciences.558

Rummun, N., Somanah, J., Ramsaha, S., Bahorun, T., & Neergheen-Bhujun, V. S. (2013). Bioactivity of nonedible parts of *Punica granatum* L.: A potential source of functional ingredients. *International Journal of Food Science*. , Article 602312. https://doi.org/10.1155/2013/602312

Sadare, O. O., & Daramola, M. O. (2019). Adsorptive removal of dibenzothiophene from petroleum distillates using pomegranate leaf (*Punica granatum*) powder as a greener adsorbent. *Chemical Engineering Communications, 206*(3), 333–345. https://doi.org/10.1080/00986445.2018.1488691

Salwe, K. J., & Sachdev, D. (2014). Evaluation of antinociceptive and anti-inflammatory effect of the hydroalcoholic extracts of leaves and fruit peel of *P. Granatum* in experimental animals. *Asian Journal of Pharmaceutical and Clinical Research, 7*(2), 137–141.

Sanna, C., Marengo, A., Acquadro, S., Caredda, A., Lai, R., Corona, A., … Esposito, F. (2021). *In vitro* anti-HIV-1 reverse transcriptase and integrase properties of *Punica granatum* L. leaves, bark, and peel extracts and their main compounds. *Plants, 10*(10), 2124. Retrieved from https://www.mdpi.com/2223-7747/10/10/2124.

Saratale, R. G., Shin, H. S., Kumar, G., Benelli, G., Kim, D.-S., & Saratale, G. D. (2018). Exploiting antidiabetic activity of silver nanoparticles synthesized using *Punica granatum* leaves and anticancer potential against human liver cancer cells (HepG2). *Artificial Cells, Nanomedicine, and Biotechnology, 46*(1), 211–222. https://doi.org/10.1080/21691401.2017.1337031

Sarkar, S., & Kotteeswaran, V. (2018). Green synthesis of silver nanoparticles from aqueous leaf extract of pomegranate (*Punica granatum*) and their anticancer activity on human cervical cancer cells. *Advances in Natural Sciences: Nanoscience and Nanotechnology, 9*(2), Article 025014. https://doi.org/10.1088/2043-6254/aac590

Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y., Zhang, Y., Nair, M. G., & Heber, D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of Nutritional Biochemistry, 16*(6), 360–367. https://doi.org/10.1016/j.jnutbio.2005.01.006

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum* L.) peel: A review. *Food Chemistry, 261*, 75–86. https://doi.org/10.1016/j.foodchem.2018.04.039

Singh, R. P., Chidambara Murthy, K. N., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) Peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry, 50*(1), 81–86. https://doi.org/10.1021/jf010865b

Swilam, N., & Nematallah, K. A. (2020). Polyphenols profile of pomegranate leaves and their role in green synthesis of silver nanoparticles. *Scientific Reports, 10*(1), 14851. https://doi.org/10.1038/s41598-020-71847-5

Toda, K., Ueyama, M., Tanaka, S., Tsukayama, I., Mega, T., Konoike, Y., … Suzuki-Yamamoto, T. (2020). Ellagitannins from *Punica granatum* leaves suppress microsomal prostaglandin E synthase-1 expression and induce lung cancer cells to undergo apoptosis. *Bioscience, Biotechnology, and Biochemistry, 84*(4), 757–763. https://doi.org/10.1080/09168451.2019.1706442

Trabelsi, A., El Kaibi, M. A., Abbassi, A., Horchani, A., Chekir-Ghedira, L., & Ghedira, K. (2020). Phytochemical study and antibacterial and antibiotic modulation activity of *Punica granatum* (pomegranate) leaves. *Scientifica, 2020*, Article 8271203. https://doi.org/10.1155/2020/8271203

Uysal, S., Zengin, G., Aktumsek, A., & Karatas, S. (2016). Chemical and biological approaches on nine fruit tree leaves collected from the Mediterranean region of Turkey. *Journal of Functional Foods, 22*, 518–532. https://doi.org/10.1016/j.jff.2016.02.006

Vidovix, T. B., Quesada, H. B., Bergamasco, R., Vieira, M. F., & Vieira, A. M. S. (2021). Adsorption of Safranin-O dye by copper oxide nanoparticles synthesized from *Punica granatum* leaf extract. *Environmental Technology*, 1–17. https://doi.org/10.1080/09593330.2021.1914180

Villis, P. C. M., de Macedo, A. T., Furtado, H. L. A., Fontenelle, P. H. C., Gonçalves, I. S., Mendes, T. L., … Santos, J. R. A. (2021). A study of the disruptive effect of the acetate fraction of *Punica granatum* extract on *Cryptococcus* biofilms. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.568258

Viswanatha, G. L., Venkataranganna, M. V., & Prasad, N. B. L. (2019). Methanolic leaf extract of *Punica granatum* attenuates ischemia-reperfusion brain injury in Wistar rats: Potential antioxidant and anti-inflammatory mechanisms. *Iranian Journal of Basic Medical Sciences, 22*(2), 187.

Viswanatha, L., Venkataranganna, V., Prasad, L., & Ashok, G. (2016). Evaluation of anti-epileptic activity of leaf extracts of *Punica granatum* on experimental models of epilepsy in mice. *Journal of Intercultural Ethnopharmacology, 5*(4), 415–421. https://doi.org/10.5455/jice.20160904102857

Willett, W., Rockstrom, J., & Loken, B. (2020). Food in the Anthropocene: The EAT-lancet commission on healthy diets from sustainable food systems. *The Lancet, 393*(10170), 447–492.

Wmank, W., & Perera, B. G. K. (2016). Preparation of medicinal soap products using the leaf extracts of *Punica granatum* (pomegranate). *International Journal of Pharma and Bio Sciences, 6*(2), 7–16.

Xiang, L., Xing, D., Lei, F., Wang, W., Xu, L., Nie, L., & Du, L. (2008). Effects of season, variety, and processing method on ellagic acid content in pomegranate leaves. *Tsinghua Science and Technology, 13*(4), 460–465. https://doi.org/10.1016/S1007-0214(08)70074-9

Xueli, W., Dongming, X., Yi, D., Yunyun, C., Zhen, M., & Lijun, D. (2005). Determination and pharmacokinetic study of brevifolin in rat after ig administration of pomegranate leaf extract. *Chinese Pharmacological Bulletin, 21*(3), 369–372.

Yu, M., Gouvinhas, I., Pires, M. J., Neuparth, M. J., Costa, R. M. G.d., Medeiros, R., … Oliveira, P. A. (2023). Study on the antineoplastic and toxicological effects of pomegranate (*Punica granatum* L.) leaf infusion using the K14-HPV16 transgenic mouse model. *Food and Chemical Toxicology, 174*, Article 113689. https://doi.org/10.1016/j.fct.2023.113689

Yu, M., Gouvinhas, I., Rocha, J., & Barros, A. I. R. N. A. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports, 11*(1), 10041. https://doi.org/10.1038/s41598-021-89437-4

Yu, X., Wang, X.-P., Lei, F., Jiang, J.-F., Li, J., Xing, D.-M., & Du, L.-J. (2017). Pomegranate leaf attenuates lipid absorption in the small intestine in hyperlipidemic mice by inhibiting lipase activity. *Chinese Journal of Natural Medicines, 15*(10), 732–739. https://doi.org/10.1016/S1875-5364(17)30104-8

Zare, H., Amiri Ardekani, E., Tavakoli, A., Bradley, R., Tavakoli, F., & Pasalar, M. (2023). Reporting of adverse effects of pomegranate in clinical studies: a systematic review. *Journal of Complementary and Integrative Medicine*. https://doi.org/10.1515/jcim-2022-0247

Zarfeshany, A., Asgary, S., & Javanmard, S. H. (2014). Potent health effects of pomegranate. *Advanced Biomedical Research, 3*(100), 1–8. https://doi.org/10.4103/2277-9175.129371

Zhang, L., Gao, Y., Zhang, Y., Liu, J., & Yu, J. (2010). Changes in bioactive compounds and antioxidant activities in pomegranate leaves. *Scientia Horticulturae, 123*(4), 543–546. https://doi.org/10.1016/j.scienta.2009.11.008