

• Review

Phytochemical profile and biological activity of *Juglans regia*

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ABSTRACT

Juglans regia Linn. (Juglandaceae), popularly known as English or Persian walnut, is a valuable medicinal plant with a potency to cure various diseases in traditional medicine. Since ancient time, different local ethnic groups have used various part of *J. regia* for a wide array of ailments including helminthiasis, diarrhea, sinusitis, stomach ache, arthritis, asthma, eczema, scrofula, skin disorders, diabetes mellitus, anorexia, thyroid dysfunction, cancer and infectious diseases. Biological activities of *J. regia* have been reported in several peer review journals and scientific attention is increasing. The present review attempts to provide comprehensive information on plant description, ethnobotanical use, toxicity, phytochemical profile, pharmacology, clinical studies and current research prospective of the *J. regia*. Currently, there is an immense interest on isolation/identification of active constituents from walnut and screening those active compounds for pharmacological activities. In addition, researchers are performing clinical trials as well as screening various solvent extracts or fractions of *J. regia* in several animal diseases models to identify promising therapeutic benefits. In the present work, we review the latest information based on published scientific investigations of *J. regia*.

Keywords: plant extracts; *Juglans regia*; medicine, traditional; phytochemicals; pharmacology

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1 Introduction

It is well accepted that herbs serve as a rich source of medicines for various ailments. However, the ethnopharmacological uses of these medicinal plants may vary due to the principles of individual traditional medicine system and formulations prepared by local ethnic groups. For example, the Ayurvedic system is most popular in south Asian countries like India and Nepal; acupuncture and traditional Chinese medicine are practiced in China, Korea and Japan; the Unani system is practiced mostly in Middle-East countries^[1]. At present, these different systems of alternative medicine practice

are flourishing worldwide. Therefore, it is essential to compile a database of evidence-based scientific information pertaining to the use of herbal medicines, their identification features, abundance of phyto-constituent, indication of medical use, harmful effects and other characteristics. Each plant yields secondary metabolites, including compounds like essential oils, phenolic compounds, terpenoids, alkaloids, steroidal compounds, glycosides, terpenes, and tannins, which may be responsible for various therapeutics effects. These plant constituents possess numerous favourable physiological properties, such as antioxidant, anti-inflammatory and anti-atherosclerotic^[2–4]. Various parts

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of *Juglans regia*, English or Persian Walnut, containing potent chemical constituents, have been continuously used since antiquity to treat diverse ailments, including diarrhoea, hyperglycaemia, cancer, infectious disease, anorexia, eczema, asthma, helminthiasis, arthritis, sinusitis, stomach-ache, skin disorders, among others. This review focuses on the most recent information on the pharmacological and phytochemical profiling of *J. regia*.

2 Ethnobotanical use

The leaves of *J. regia* are popular in complementary and alternative therapy as antimicrobial, depurative, keratolytic, antidiarrheal, carminative, anthelmintic, astringent, tonic, hypoglycaemic and for the treatment of sinusitis, cold and stomachache^[5-7]. In Turkish folk medicine, fresh leaves of *J. regia* are used on the forehead and body to alleviate fever and on joints to reduce pain from rheumatism^[8,9]. Similarly, in Iranian traditional medicine, the kernel from *J. regia* has been used for colitis^[10]. In Palestine, *J. regia* has been used to treat diabetes, cardiac disease and inflammatory conditions^[11,12], as well as to improve vascular and prostate health in elderly males^[13]. In China, the bark from branches and exocarp from the immature, green fruit have been used for the treatment of gastric, liver and lung cancer^[14,15]. In Mexico, it is used by traditional healers for protection from liver damage^[16]. In Nepal, the bark paste is used to

treat arthritis, skin diseases, toothache and to promote hair growth; the seed coat is used for healing wounds^[17]. In Calabrian indigenous medicine, the shell of *J. regia* is used to treat malaria^[18].

3 Reported phytochemicals

3.1 Steroids

The steroidal phytochemicals reported in *J. regia* are shown in Figure 1^[19,20].

3.2 Flavonoid C-glycoside

The flavonoid C-glycoside phytochemicals reported in *J. regia* are shown in Figure 2^[21].

3.3 Flavones

The flavones reported in *J. regia* are shown in Figure 3^[19,22].

3.4 Essential oil component

The essential oil component phytochemicals reported in *J. regia* are shown in Figure 4^[19,20,23-25].

3.5 Tannins

The tannins reported in *J. regia* are shown in Figure 5^[26].

3.6 Miscellaneous

Organics acids: The organics acids reported in *J. regia* are shown in Figure 6^[22,27-29].

Phenolic aldehyde: The phenolic acids reported in *J. regia* are shown in Figure 7^[28,30].

Monoglyceride: The monoglyceride reported in *J. regia* is shown in Figure 8^[30].

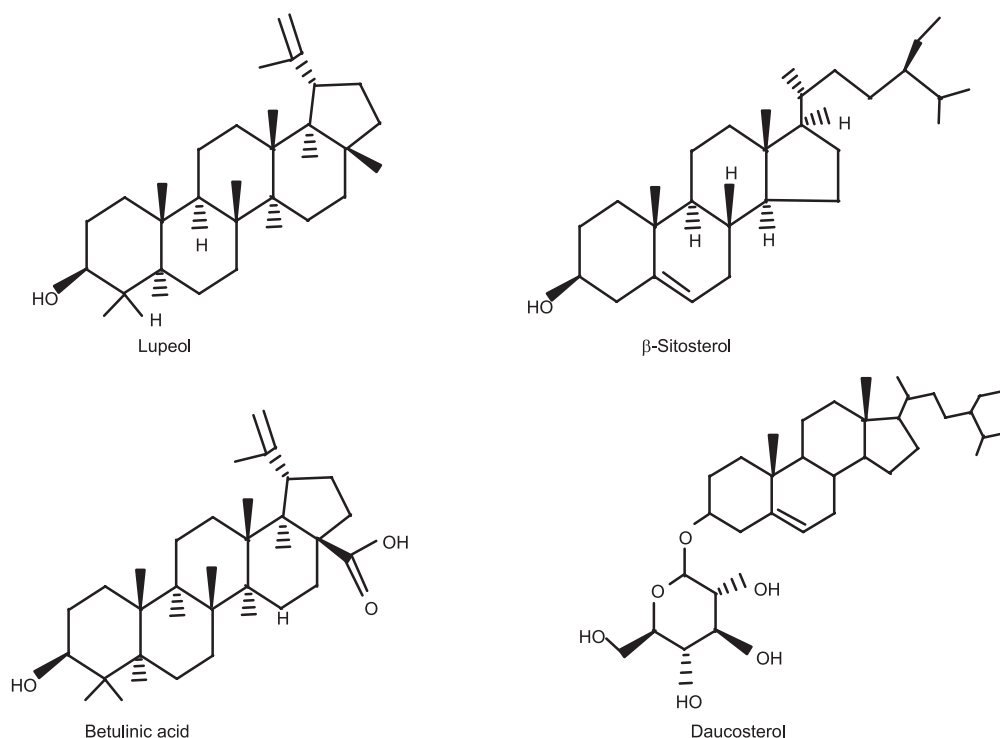


Figure 1 Steroidal chemical constituents present in *Juglans regia*

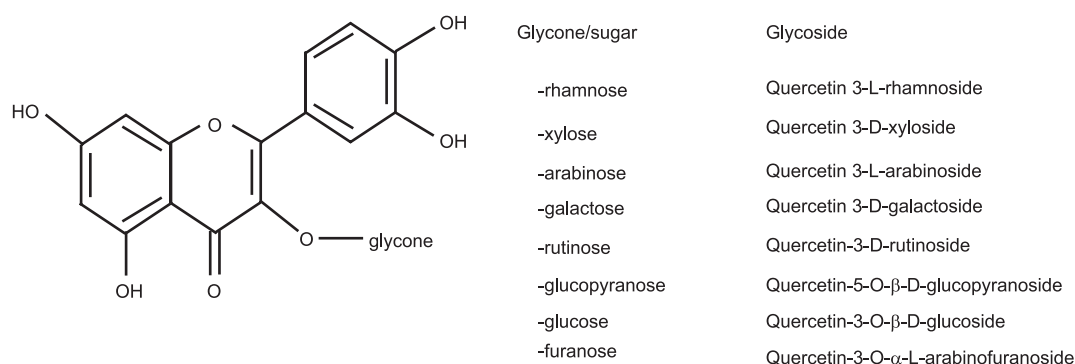


Figure 2 Flavonoid C-glycoside present in *Juglans regia*

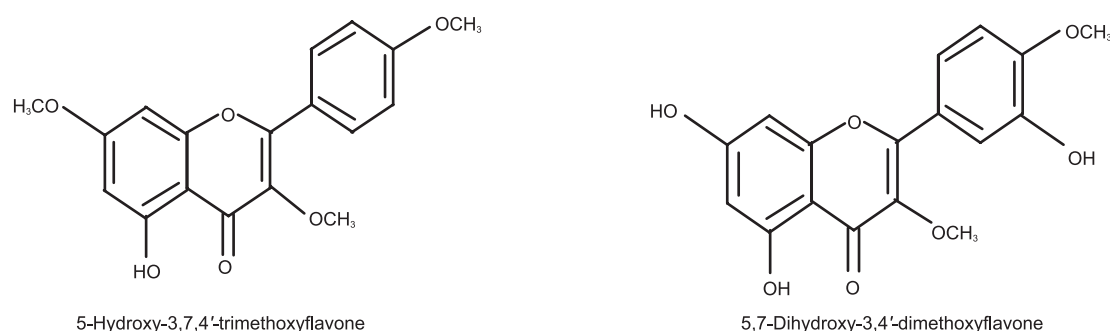


Figure 3 Flavones present in *Juglans regia*

Sesquiterpene: The sesquiterpene reported in *J. regia* is shown in Figure 9^[31].

Diarylheptanoid: The diarylheptanoids reported in *J. regia* are shown in Figure 10^[31,32].

4 Pharmacology

4.1 Antioxidant activity

The essential oil from *J. regia* leaves was analysed for its antioxidant potency using 2 different *in vitro* assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging. Results showed a high antioxidant capacity with 50% inhibiting concentration (IC₅₀) values of 34.5 and 56.4 µg/mL respectively^[21]. In an *in vivo* rat model of oxidative stress, administration of walnut leaf extract at doses of 0.2 to 0.4 g/kg body weight for 4 weeks protected the animals from hepatic damage caused by carbon tetrachloride (CCl₄). Moreover, the walnut extract elevated antioxidant enzymes: superoxide dismutase and catalase^[33]. Major flavonoids like quercetin-3-*O*-rhamnoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-xyloside, kaempferol-*O*-pentoside derivative, quercetin-3-*O*-galactoside and kaempferol-*O*-pentoside isolated from *J. regia* leaves showed noteworthy radical-scavenging activities by reducing the reactive oxygen species (ROS) level in RAW264.7 cells^[34]. Flavonoids exhibited potent

antioxidant activity and have been useful in regulating immune function and improving anticancer activity^[34]. Haddad *et al*^[35] investigated the benefit of consuming walnuts on food-mediated oxidative stress in healthy volunteers. The level of plasma malondialdehyde, a marker of lipid peroxidation, was attenuated by 7.4% after consumption of walnut meal, whereas hydrophilic and lipophilic oxygen radical absorbance capacity was raised by 7.5% and 8.5% respectively. Furthermore, the oxidation of low-density lipoprotein (LDL) was halted 2 h after consumption of walnut meal. Similarly, the level of antioxidant components from walnut, epicatechin gallate, gallic acid, gallic acid gallate and epigallocatechin gallate, were raised significantly 1 h after walnut meal consumption. Likewise, the amount of urolithin-A (a metabolic product of ellagic acid derivatives) elimination *via* urine was remarkably higher after consumption of walnut meal. In this study, the walnut meal successfully attenuated oxidative stress compared to a refined control diet^[35]. Leaves of walnut obtained from 14 different sources were investigated for their antioxidant potency and the availability of total phenolic compounds. From the different sources, total phenolic content ranged from 17.7–39.6 mg gallic acid equivalents (GAE)/g. The methanolic extract possessed the most potent DPPH-scavenging activity^[36]. Using a high-speed counter current

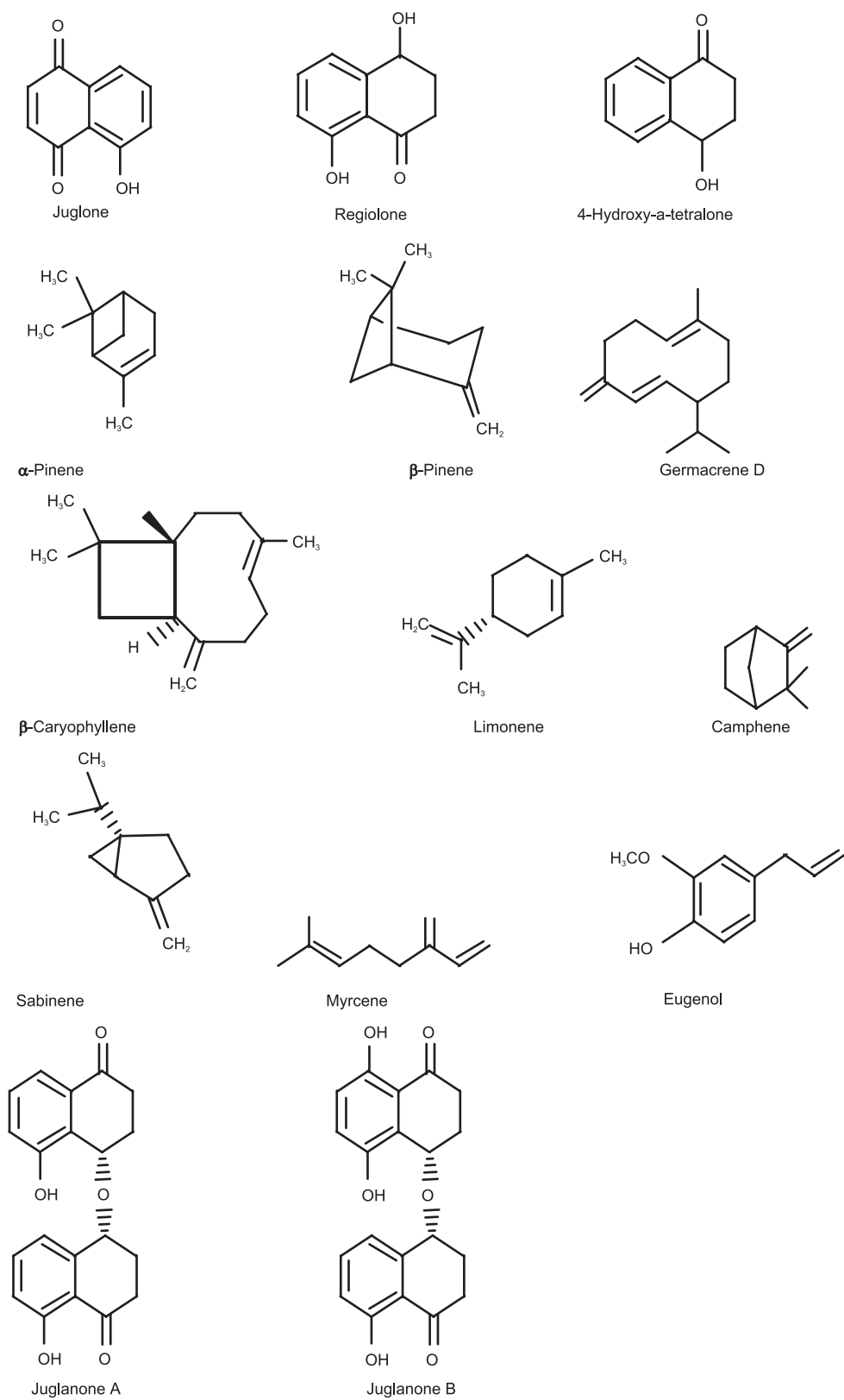


Figure 4 Essential oil components present in *Juglans regia*

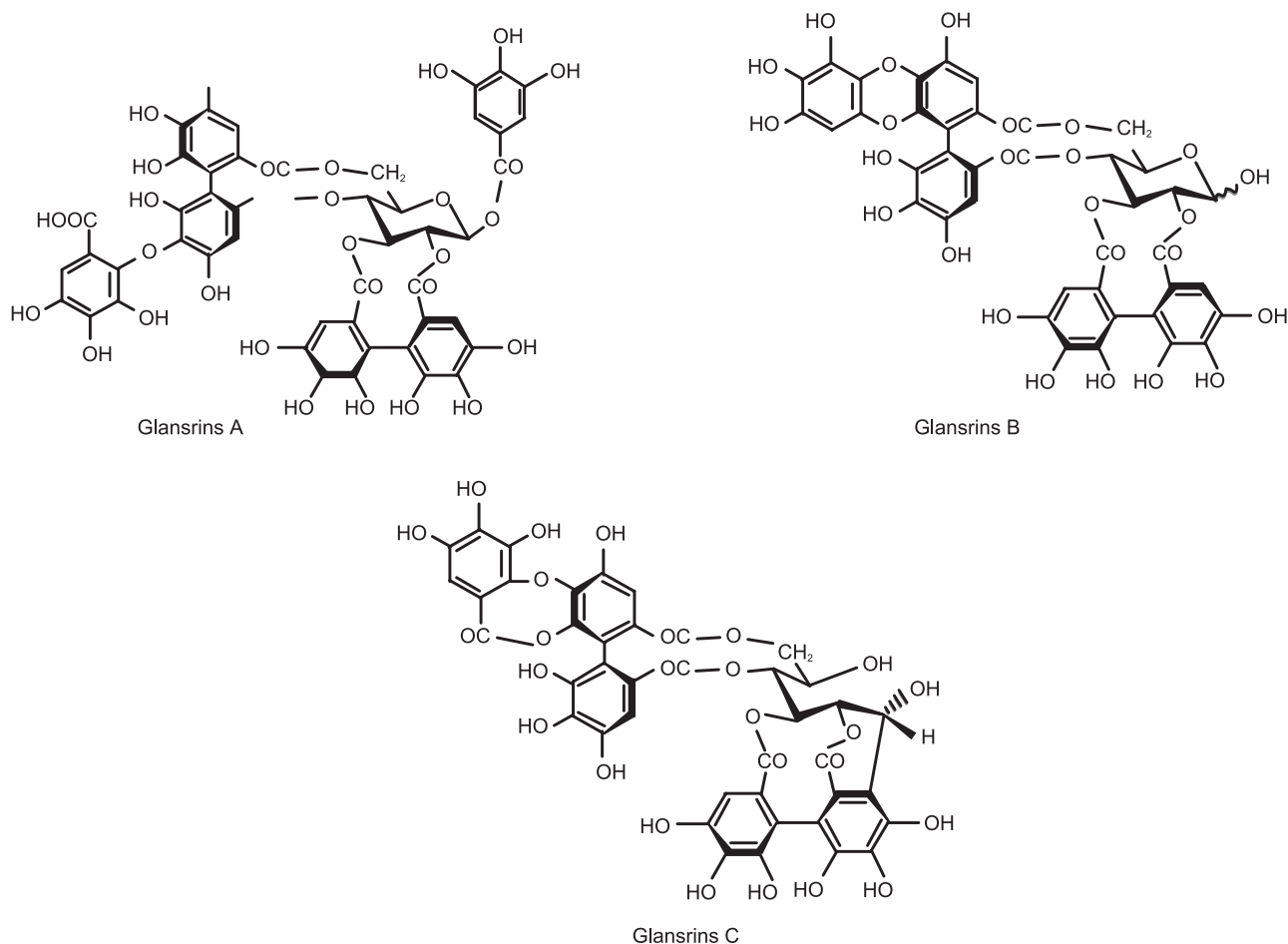


Figure 5 Tannins present in *Juglans regia*

chromatography method, Grace *et al*^[37] isolated various compounds from the butanol and ethyl acetate fractions of walnut extract and tested their capacity to inhibit lipopolysaccharide (LPS)-stimulated ROS generation in human neuroblastoma cells and to inhibit lipid accumulation in 3T3-L1 mouse adipocytes. The isolated ellagitannins reduced ROS formation in SH-SY5Y cells, whereas 2,3-*O*-hexahydroxydiphenoyl glucopyranoside at 90 $\mu\text{mol/L}$ inhibited lipid accumulation dose-dependently^[37]. Carey *et al*^[38] investigated the beneficial ability of polyunsaturated fatty acids (PUFAs) from walnut extract to slow down the damage of hippocampal cells, mediated by inflammation and oxidative stress. The outcome of this study revealed that walnut extract delivered noteworthy defence against cell death and calcium dysregulation in a concentration-dependent manner.

4.2 Cognitive function

Aging is associated with a decrease in performance of motor and cognitive function; polyphenols in nuts and fruits can reduce this deficit. Walnuts are a rich source

of antioxidant polyphenols and lipids. Willis *et al*^[39] studied the advantage of feeding a 2%–9% walnut diet on motor function and cognitive capacity in 19 month-old rats. Results for motor testing were dependent on the percentage of walnut in the animals' diet. There was an improvement on rod walking ability for the group fed the 2% walnut diet, whereas progress on the medium plank walk performance by the 6% walnut diet. The results of the Morris water maze (MWM) showed that all (2%–9%) of the walnut diets enhanced working memory, however there was impairment in reference memory in the 9% diet group. This study revealed that moderate dietary supplementation of walnut could be beneficial to improve motor performance and cognitive skills of older rats^[39]. There are significant amount of PUFAs in *J. regia*, which are considered favourable for maintaining brain health. Polyphenolic constituents available in walnuts are capable of exhibiting antioxidant activity, reducing inflammatory burden on brain cells, improving signalling pathway between neurons, increasing neuron generation and boosting the removal of insoluble harmful protein

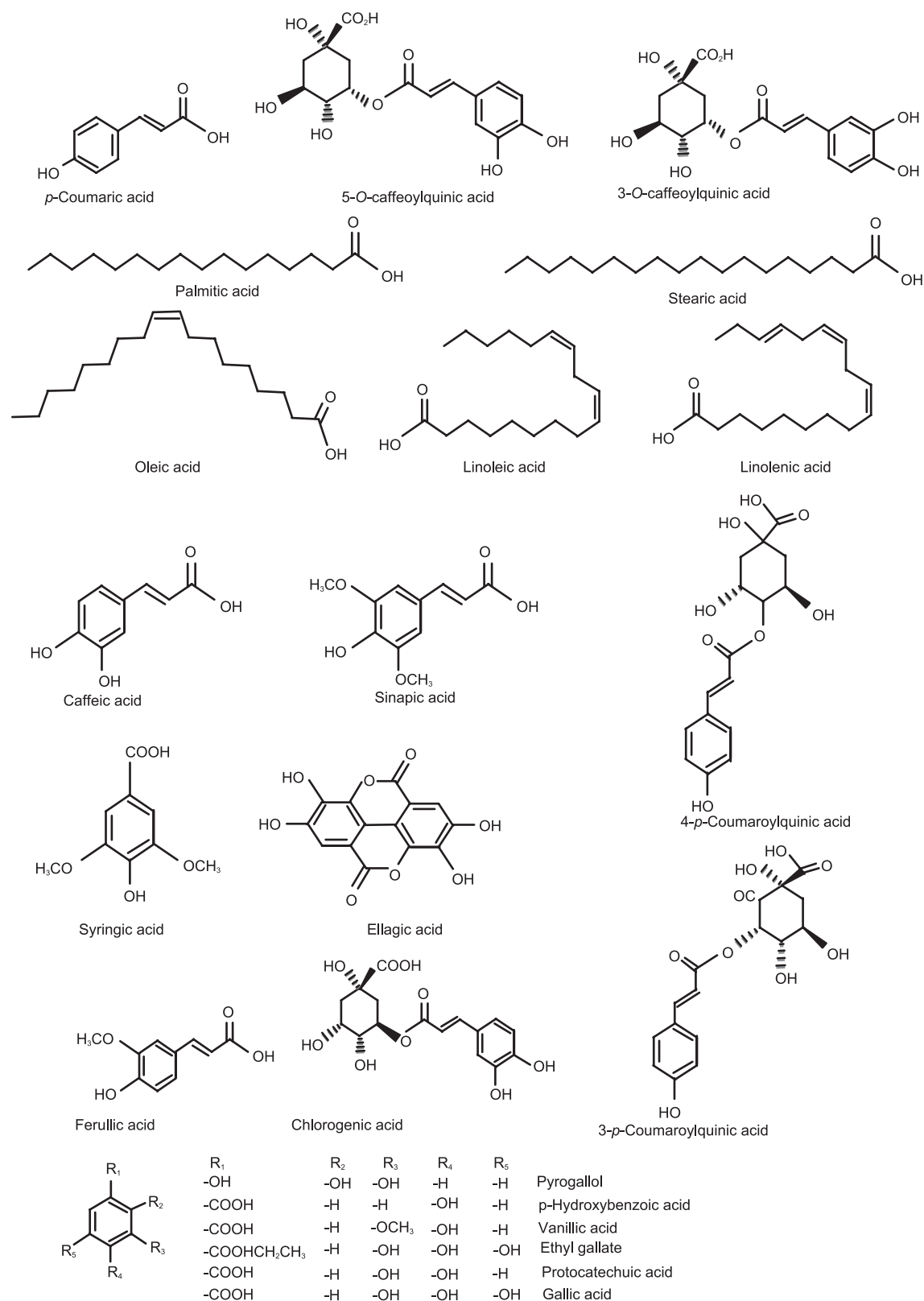


Figure 6 Organic acids present in *Juglans regia*



accumulates^[40]. In comparison to normal diet, rats fed with 80 mg/d of walnuts for 28 d showed enhanced learning and memory potential in the radial-arm maze and also had reduced anxiety on the elevated plus maze^[41]. One of the main reasons for walnut being beneficial for cognitive function is thought to be its PUFA composition. On average, walnuts contain 38.1 g of C18:2n-6 linoleic acid (LA) and 9.08 g of C18:3n-3 α -linolenic acid (ALA) in 100 g of walnuts. LA and ALA are considered essential fatty acids because they are necessary for health, but

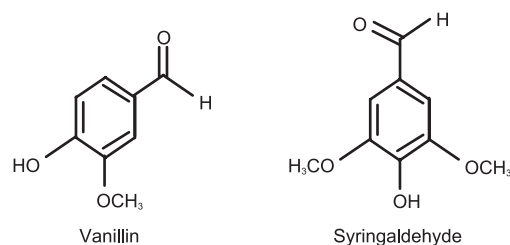


Figure 7 Phenolic acids present in *Juglans regia*

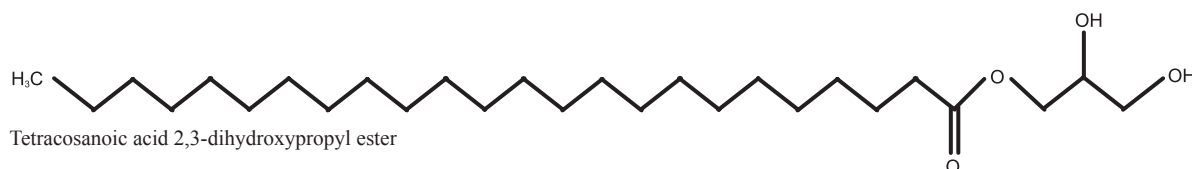


Figure 8 Monoglyceride present in *Juglans regia*

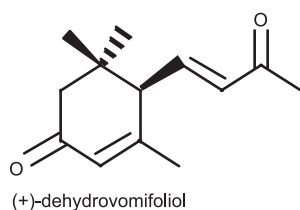


Figure 9 Sesquiterpene present in *Juglans regia*

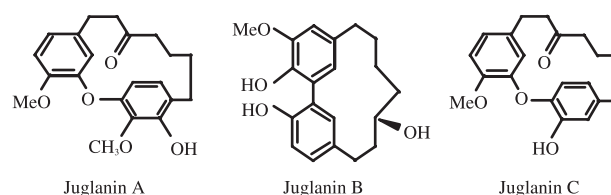


Figure 10 Diarylheptanoids present in *Juglans regia*

cannot be synthesized by humans and therefore must be consumed in the diet. Declines in cognitive and motor functions during aging are predominantly due to nerve injury and malfunction in response to diverse factors, such as oxidative stress and alteration in lipid composition of the brain. Contemporary *in vivo* animal studies and human clinical trials have highlighted the importance of dietary intervention to slow down the cognitive aging. These cognitive, memory and behavioural deterioration can be mitigated with daily intake of foods, like walnuts, containing PUFAs and polyphenols. PUFA is beneficial during aging, as it can ameliorate the cholinergic signalling in the nervous system, perhaps resulting in improved cognitive function^[42–44]. A study of pregnant and lactating rats revealed that feeding mothers with walnut kernel resulted in improvements in memory and learning of the adult pups, according to the MWM test^[45].

4.3 Neuroprotective

Excessive production of ROS within the brain is a key reason for decline of neural capacity and cognitive function. Fisher *et al*^[46] reported that walnut serum inhibited LPS-mediated nitrite production in BV-2 microglia of old mice. Furthermore, there was notable inhibition of tumour necrosis factor- α

(TNF- α), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2, mediators of inflammation, suggesting the protective effect of walnut. Shabani *et al*^[47] studied the neuroprotective activity of a 6% walnut diet against neurotoxicity in male rats induced by the anticancer drug cisplatin. The results showed that administration of walnut improved the cognitive and motor functions, demonstrating the potential benefits of including walnut in the diet for combating chemotherapy-induced disruptions of motor and cognitive function^[47]. Similarly, a study done by Essa *et al*^[48] highlighted the efficacy of a walnut diet for neuro-protection in mice with Parkinson's disease. Consumption of a walnut diet reversed the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-mediated neurotoxicity. This result may have been due to antioxidant activity, MAO-B inhibition, or preservation of mitochondrial function, suggesting the beneficial role of walnut in Parkinson's disease^[48]. Extracts of *J. regia* fruits and leaves exhibited low inhibition of butyrylcholinesterase, and no significant inhibition of acetylcholinesterase^[49].

4.4 Diabetes and complications

Various *in vivo* animal and human studies have shown antidiabetic activity of *J. regia* products. In rats with alloxan-induced diabetes, *J. regia* leaf extract administered

at a dose of 200 mg/kg, demonstrated antidiabetic activity similar to glibenclamide (0.6 mg/kg), a standard drug for hyperglycemia. *J. regia* extract also reduced fasting blood glucose and glycosylated haemoglobin (HbA1c) levels, whereas it led to increased insulin levels compared to untreated diabetic rats^[50]. *J. regia* leaf extract showed similar results in another rat model of diabetes induced by streptozotocin-nicotinamide^[51]. In a clinical study, patients with type 2 diabetes, who were given *J. regia* leaf extract (100 mg capsule) twice daily for a period of 3 months, showed reduced fasting blood glucose, HbA1c, triglycerides and total cholesterol levels relative to the placebo group^[52]; patients had no adverse side effects. In another human study from Iran, patients with type 2 diabetes, receiving aqueous extract of *J. regia* leaf for 2 months, had reduced blood glucose and serum HbA1c levels and significantly increased insulin levels^[53]. In mouse myoblast cell line C2C12, methanolic extract of *J. regia* leaves, at a dose of 25 µg/mL, enhanced the glucose uptake rate and halted the protein tyrosine phosphatase 1B expression, providing *in vitro*-based rationale indicating a potential for use against elevated blood glucose levels^[54]. In a randomized crossover study, it was found that consumption of walnut-enriched meal raised plasma adiponectin levels, suggesting a beneficial role of walnut in increasing postprandial adiponectin^[55].

4.5 Anticancer

Juglone, obtained from the chloroform extract of *J. regia* root, and its synthetic triazolyl analogues showed cytotoxic activity against various human cancer cell lines, such as lung (NCI-H322 and A549), breast (T47D), skin (A-431), prostate colon (Colo-205 and HCT-116), and prostate (PC-3 and DU-145). Surprisingly, synthetic triazolyl derivatives had greater cytotoxicity, but their effects were specific to lung cancer cell lines. Some analogues even demonstrated higher activity against NCI-H322 and A549 cancer cell lines than the commercial anticancer agent BEZ235 (a phosphoinositide 3-kinase and mTOR dual inhibitor)^[56]. Likewise, the treatment with green walnut husks and root bark extract of *J. regia* induced apoptosis in the human breast cancer cell line MDA-ME231 and prostate cancer cell line PC-3 by up-regulating caspase 3, caspase 8 and Bax gene expression and down-regulating Bcl-2 gene expression^[57,58]. An *in vivo* experiment was conducted, comparing the effect of walnut, flaxseed and corn oil separately on colorectal cancer in female nude mice, induced by injecting HT-29 human colon cancer cells. Compared to the control groups fed with corn oil, the rats given walnut and flaxseed for 25 d showed a significantly slower tumour growth rate. Immunohistochemical analysis for CD34 staining showed that walnut significantly reduced angiogenesis with greater activity than flaxseed oil^[59]. Another similar

pilot study was carried out by injecting MDA-MB231 cells into nude mice and feeding them ground walnut. The tumour growth rate was significantly lower in the group fed with walnut than in the control group, which was fed 10% corn oil. Furthermore, in another experiment, the proliferation of tumour cell was reduced by diet including walnut^[60]. A walnut peptide complex with zinc (WP1-Zn) was evaluated for its anticancer property against breast cancer cell line MCF-7. WP1-Zn displayed strong action against MCF-7 cell proliferation by arresting cell cycle processes and causing apoptosis through the ROS-activated mitochondrial pathway. Also, there was a 20.02% decrease in the survival rate following a 48 h treatment of 300 µg/mL of WP1-Zn^[61]. Ma *et al*^[62] recently isolated a new biopeptide from hydrolysed walnut protein and investigated its effects on induction of apoptosis and autophagy in cancer cells. The novel purified peptide, with a sequence of CTLEW (Cys-Thr-Leu-Glu-Trp), showed significant growth inhibitory activity, as well as induction of apoptosis and autophagy on MCF-7 cells. Also, CTLEW notably halted the proliferation of HeLa and Caco-2 cells. Furthermore, the biopeptide amplified growth and IL-2 production of spleen lymphocytes, elevated nitric oxide (NO) generation and phagocytosis by macrophages, suggesting a potent activity against cancer cell proliferation as well as an immune boosting capacity. Oguzhan *et al*^[63] studied the anticancer activity of walnut milk (WM) in DU145 (human prostate cancer cell) and MCF-7 cells. Results showed a remarkable reduction in cell viability and selective induction of caspase-dependent apoptosis in both cell lines, through stimulation of a constitutive apoptotic pathway and facilitation of ROS generation^[63]. Sánchez-González *et al*^[64] studied the function of walnut urolithins, metabolic products of ellagic acid on regulation of prostate cancer, focusing on the androgen receptor (AR). Results showed that in LNCaP (human prostate cancer) cells, the mRNA level and protein expression of both AR and prostate specific antigen (PSA) were down-regulated by urolithins. Similarly, urolithins caused apoptosis of LNCaP cells, along with a reduction in the level of Bcl-2 protein. This study supports the notion that consumption of walnuts could help reduce the risk of developing prostate cancer^[64]. The chloroform fraction of *J. regia* leaf extract showed antiproliferative activity against human oral cancer, colon adenocarcinoma and breast adenocarcinoma cell lines with IC₅₀ values ranging 0.36–0.81 mg/mL, as well as the cell cycle arrest at G₀/G₁ phase after 24-hour treatment^[65].

4.6 Cardioprotective activity

Mortality and morbidity from various cardiovascular diseases (CVDs) are regarded as a global health burden. Various factors like hypercholesterolemia^[66], endothelial dysfunction^[67] and circulating microparticles^[68] lead

to numerous CVDs. Herbal plant extracts or isolated compounds can be potent treatments to slow down CVDs such as atherosclerosis in both *in vitro* and *in vivo* models^[69–71]. The promising health benefits of walnut intake may be due to their containing fatty acids rich in PUFAs, and a beneficial ratio of $\omega 3:\omega 6$. Health promoting phytoconstituents present in walnut, including polyphenols, have been claimed to decrease the risk of CVDs^[72]. In comparison to other varieties of nuts having mainly monounsaturated fatty acid, *J. regia* is unusual, as they mainly contain PUFAs. In spite of being calorie-rich, walnut intake does not result in weight gain when taken as a food supplement. Different pathways have been outlined to explain the beneficial effects of walnuts in the control of CVD risk. Walnut can alter the serum lipoprotein levels, increase NO formation *via* increasing arginine in protein, promote endothelial function, as well as inhibit vascular smooth muscle cell proliferation, monocyte adherence, chemotaxis and platelet aggregation^[73]. Davis *et al*^[74] measured the aortic endothelin-1 (ET-1) and other CVD risk markers in hamsters given high-fat atherogenic diets and walnuts or α/γ -tocopherol for 26 weeks. The level of ET-1 mRNA was significantly reduced by walnut consumption. Similarly, cholesterol ester in the aorta was also significantly decreased in the walnut-fed group compared to the lowest α -tocopherol fed group. This study demonstrated the cardioprotective effect of walnut by targeting ET-1 expression and lowering aortic cholesterol ester^[74]. Gu *et al*^[75] hydrolysed walnut proteins to obtain a potent peptide, having the ability to block the angiotensin-I-converting enzyme (ACE). This peptide has a sequence of Tyr-Glu-Pro; its median inhibitory concentration for ACE was 0.29 $\mu\text{mol/L}$, suggesting it as a potential therapeutic option for treating conditions like hypertension^[75]. Similarly, Wang *et al*^[76] also isolated walnut protein hydrolysate through two different enzymatic methods, alcalase and trypsin. ACE inhibitory and antihypertensive activities of both trypsin-generated walnut protein hydrolysate (TWPH) and alcalase-generated walnut protein hydrolysate (AWPH) were compared. Both TWPH and AWPB showed potent ACE-blocking activity. In spontaneously hypertensive rats, the greatest reduction of systolic blood pressure by TWPH and AWPB was observed at 6 (–30 mmHg) and 4 h (–6 mmHg), respectively, after administration, revealing its antihypertensive property^[76]. An intervention study was carried out to explore the cardioprotective effect of walnut in the apolipoprotein E-knockout mouse model of atherosclerosis. Mice were given a high-fat diet (HFD) and either sunflower oil (control group), or walnuts, or walnut oil, for 8 weeks. Mice receiving whole walnut, not just walnut oil, showed a significant (55%) reduction of atherosclerotic plaque formation in

the aortic arch, with respect to the control group. This finding was consistent with the observed decrease in macrophage CD36 (scavenger receptor) staining. Walnuts also reduced the levels of plasma cholesterol, triglycerides and prothrombin (23%, 36%, and 21% respectively). Furthermore, lipid accumulation in the liver was reduced, suggesting the potential benefit of walnut consumption against atherosclerotic plaque formation and lipid metabolism^[77].

4.7 Hepatoprotective activity

In mice fed walnut pellicles (WP, 200 mg/kg), the thin skin around the nut meat, WP significantly attenuated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevation in CCl_4 -injured liver, however it did not affect D-galactosamine (GalN)-caused liver injury. Constituents like tellimagrandins I, II and rugosin C reduced damage to hepatocytes from CCl_4 , whereas tellimagrandin I and 2,3-*O*-hexahydroxy diphenyl glucose reduced damage induced by D-GalN^[78]. Walnut leaf extract (ranging from 0.2 to 0.4 g/kg body weight) showed protective effects in rats with CCl_4 -damaged liver by lowering serum ALT, AST and alkaline phosphatase levels^[33]. Jugnole, from walnuts, protected HFD-induced liver damage in rats by inhibiting various inflammatory cytokines, like TNF- α , IL-1 β and IL-6, through down-regulation of toll-like receptor 4 and activation of NF- κB activity^[79]. Similarly, Bati *et al*^[80] studied the hepatoprotective effect of various walnut doses against ethanol-induced oxidative stress in rats by evaluating the serum marker enzymes of liver injury. Serum biochemistry results revealed a significant rise in AST, gamma glutamyl transpeptidase, ALT and lactate dehydrogenase (LDH) in the 20% ethanol-fed group, compared with the control group (without ethanol). In contrast, these markers decreased in 20% ethanol + 10% walnuts-fed group, suggesting hepatoprotective effect of walnut^[80]. Ellagitannins are abundant in WP. Walnut extract containing 45% polyphenol was examined for its lipid-lowering effect in HFD-fed mice. There was a significant decrease in serum triglycerides and liver weight in mice receiving 100 and 200 mg/kg WP extract. Other effects of treatment with WP extract included, the up-regulation of β -oxidation of the cytosolic fraction of liver tissue, increased expressions of hepatic acyl coenzyme A oxidase 1 (ACOX1) and up-regulation of peroxisome proliferator-activated receptor- α (PPAR- α). Similarly, *in vitro* results on the human liver cancer cell line, HepG2, showed that the mRNA expressions of PPAR- α , carnitine palmitoyltransferase 1A and ACOX1 were also significantly increased by walnut extract. Similarly, ACOX1 expression was also augmented by tellimagrandin I (a polyphenol compound) from walnut, suggesting a hypotriglyceridemic potential of WP^[81].

4.8 Anti-inflammatory activity

Aqueous and ethanolic extracts of *J. regia* leaves were evaluated for anti-inflammatory activity. In carrageenan-induced hind paw edema in mice, only the ethanolic extract of *J. regia* showed anti-inflammatory activity, without causing gastric disturbance^[82]. BV-2 microglial cells treated with *J. regia* extract before LPS stimulation showed reduced production of NO and expression of iNOS. Also, *J. regia* extract reduced production of TNF- α . Thus, walnuts showed anti-inflammatory effects in microglia, which might be useful in the prevention and treatment of neurodegeneration^[83]. The aqueous (2.87 and 1.64 g/kg) and ethanolic (2.044 and 1.17 g/kg) extracts of *J. regia* showed antinociceptive activity in hot-plate writhing tests; the antinociceptive effect was believed to operate via non-opioid receptors. These extracts also had anti-inflammatory activity, which was shown in the xylene test^[84]. Methanolic extract of *J. regia* kernel (50 and 100 mg/kg body weight) was evaluated against cigarette smoke extract (CSE)-induced lung toxicity in rats. Prophylactic administration of methanolic extract to rats (by gavage) 1 week before CSE exposure significantly reduced LDH activity, total cell count and total protein, and increased the glutathione level in bronchoalveolar lavage fluid. It also significantly restored the levels of glutathione reductase and, catalase and reduced the xanthine oxidase (XO) activity in lung tissue^[85]. Walnut methanolic extract (10–200 μ g/mL) and ellagic acid (an important compound in walnut; 10^{-7} – 10^{-5} mol/L) decreased TNF- α -induced expression of vascular cell adhesion molecule-1 and intracellular cell adhesion molecule, thus preventing an early step in inflammation^[86]. Extract of *J. regia* kernel was evaluated for its beneficial action against lung inflammation induced by CSE in rats. Common markers of lung injury, like total cell count, LDH activity and total protein, were reduced, while glutathione level was increased in bronchoalveolar lavage fluid. Furthermore, the catalase and glutathione reductase level was restored while XO activity was decreased in lung tissue by treatment with *J. regia* extract. The high polyphenol level (96.00 ± 0.81 mg GAE/g dry weight) in *J. regia* kernel extract was found to be responsible for potent DPPH radical scavenging activity by researchers exploring the beneficial role of *J. regia* extract against CSE-induced acute inflammation^[85]. Xiao *et al*^[87] highlighted the *in vivo* antihyperuricemic effect of 95% ethanol extract of walnut shell (WSE) in male SD rats and isolated the active constituents that possessed enhanced XO inhibitory activity. The *in vivo* results showed that WSE reduced the uric acid levels in serum^[87].

4.9 Antibacterial

Aqueous extracts of *J. regia* green husks suppressed gram-positive bacteria growth, where *Staphylococcus*

aureus was the most susceptible bacteria with minimal inhibitory concentration (MIC) of 0.1 mg/mL^[88]. Juglone inhibited 3 key enzymes from *Helicobacter pylori*, cystathionine γ -synthase (HpCGS), HpFabD (malonyl-CoA:acyl carrier protein transacylase) and β -hydroxyacyl-ACP dehydratase (HpFabZ), with IC₅₀ values of (7.0 ± 0.7), (20 ± 1) and (30 ± 4) μ mol/L respectively^[89]. The hexane extract of *J. regia* bark showed *in vitro* anti-*Mycobacterium tuberculosis* activity, with a MIC of 100 μ g/mL^[90]. The antimicrobial activities of *J. regia* leaf extract against *Propionibacterium acnes*, *S. aureus* and *S. epidermidis* were found with zones of inhibition ranged from 15.8–17.6, 11.3–15.7, 12.9–15.5 mm respectively^[91]. The volatile oil obtained from *J. regia* leaves and its key constituents (β -pinene, α -pinene, limonene, germacrene D and β -caryophyllene) displayed strong activity against *S. epidermidis*, *Salmonella typhi*, *S. aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Interestingly, gram-negative bacteria were less susceptible than gram-positive bacteria^[21]. Mahmoud *et al*^[92] studied the activity of walnut leaf ethanol extract against the microorganisms that cause dental plaque and found the extract was most potent against *Streptococcus salivarius* and *Streptococcus sanguinis*, based on MIC and minimum bactericidal concentration values^[92]. A comparison between ethanol extract of walnut leaf and chlorohexidine mouth rinse against *Streptococcus mutans* and *S. sanguinis* found that the activity of the two treatments were comparable against *S. sanguinis*^[93]. Similarly, Zhang *et al*^[94] studied the antimicrobial property of walnut green husk extract by the Oxford Cup method. The ethyl acetate and chloroform extracts exhibited antimicrobial activities against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*. The petroleum ether extract showed anti-fungal activity with a MIC of 3.13, 6.25 and 6.25 mg/mL for *Penicillium italicum*, *Canidia albicans* and *Penicillium digitatum*, respectively.

4.10 Clinical study

Sixty hyper-lipidemic subjects in southern Iran were divided into 2 groups (walnut and placebo) and lipid profiles were measured. Walnut oil, in 500 mg capsules, given to patients at a dose of 3 g/d, for a period of 45 d, reduced plasma triglyceride concentration by 19% to 33% of baseline ($P < 0.05$)^[95]. Hypercholesterolemic individuals ($n=21$) were given a cholesterol-reducing Mediterranean diet or walnut-enriched (100 g/d) diet. Compared to the Mediterranean diet, the walnut diet improved endothelium-derived vasorelaxation and reduced vascular cell adhesion molecule-1 expression. Walnut consumption significantly decreased the concentration of total cholesterol and LDL cholesterol^[96]. Walnut (75 g/d) given for 8 weeks to study participants ($n=21$),

significantly decreased the α -tocopherol to γ -tocopherol ratio, increased serum γ -tocopherol and increased the ratio of free PSA to total PSA, suggesting that walnuts might improve prostate and vascular health^[13]. In an *in vivo* study of Japanese women and men, a walnut diet (44–58 g/d) was given to healthy volunteers ($n=20$ each gender) for 4 weeks. Serum apolipoprotein B and total cholesterol levels were significantly reduced in walnut-fed individuals relative to reference diet-fed individuals. Although, the ratio of LDL to high-density lipoprotein (HDL) cholesterol was significantly decreased in both men and women, the LDL cholesterol level itself was significantly lowered in women only. In both sexes, the walnut diet caused elevation of serum α -linolenic acid. Also, the diet did not alter LDL oxidizability in women. Thus, α -linolenic acid present in walnut might be the reason for the observed decrease in LDL cholesterol^[97]. Female volunteers were given walnut (43 g/d) first and then a Western-type (control) diet or *vice versa*, with each lasting 8 weeks. Compared to the control diet, walnut significantly decreased non-HDL cholesterol and apolipoprotein B levels. The walnut diet also reduced total cholesterol. However, the walnut diet did not show significant differences in the levels of fasting very low-density lipoprotein, LDL, HDL, triglyceride, glucose, insulin, HOMA-IR or HbA1c. Also, the levels of fasting adipokines, C-reactive protein, biomarkers of endothelial dysfunction, postprandial lipid, glucose metabolism and endothelial function were unaffected^[98].

Plants and natural products have been used since the early days of humankind for promoting health and are still used in the modern world. Herbal medicine is safe and affordable when compared to modern allopathic medicines. Thus, currently, these traditional ethnomedicines are studied by researchers around the world with great interest^[99–101]. If benefits from these natural products can be demonstrated scientifically, then the benefits should be utilized for the promotion of public health and the prevention and treatment of diseases.

4.11 Toxicity

Previous studies have reported irritation and skin hyperpigmentation associated with topical walnut use. Although, in guinea pigs, it has been reported to be a strong sensitizer, in humans contact allergy is very rare^[102,103]. In a case report, the palms and fingers of a 65 year-old woman showed large blisters and skin hyperpigmentation following the consumption of 15 kg of walnuts in 3 d^[104]. Walnut aqueous extract modulated the toxicity of cyclophosphamide and protected metabolizing and antioxidant enzymes during the chemotherapy^[105]. In mice, the half-maximal lethal dose (LD_{50}) of intraperitoneal injection of *J. regia* leaf aqueous and ethanolic extract was found to be 5.5 and 3.3 g/kg,

respectively^[84]. According to Calabrò *et al*^[106], juglone induced the eryptosis (suicidal death of erythrocyte) by increasing the abundance of ceramide, and reducing energy level and protein kinase C (PKC) activation. In this study, treatment of human erythrocytes with juglone for 24 h, at a dose of 5 μ mol/L, caused significant reduction on erythrocyte forward scatter. Furthermore, juglone at doses from 1–5 μ mol/L significantly raised the annexin V binding percentage. Likewise, at a dose of 5 μ mol/L, juglone significantly reduced the concentration of ATP in erythrocytes, and also increased the abundance of ceramide at the surface of erythrocytes. In contrast, the activity of 10 μ mol/L juglone on binding of annexin V was considerably inhibited by removing extracellular Ca^{2+} as well as by treating of PKC inhibitor, staurosporine (1 μ mol/L)^[106]. There are also some reports of the harmful effects of walnut on animals, specifically, horses. Walnut heartwood can cause an inflammatory condition in horses called laminitis. For this reason, the black walnut extract model was developed to study the various parameters associated with laminitis in horses. This model has assisted researchers in establishing many of the initial inflammatory events that may occur in the laminitis^[107]. Chiavaccini *et al*^[108] studied the expression of calprotectin as well as apoptosis of epithelial and endothelial cells in the intestine of horses treated orally with black walnut extract. Histological analysis revealed that black walnut extract-treated horses showed eosinophil and lymphocyte epitheliotropism, indicating the initial inflammatory response experienced by the animals^[108].

5 Conclusion

The present review surveys the recent publications on walnut and its constituents. The review highlights descriptions of chemical constituents, traditional uses, pharmacological uses and toxicity studies of *J. regia*. Extensive research should be performed for structural analysis and molecular mechanism should be explored. Also, further clinical studies of phytoconstituents should be carried out to determine safety, efficacy and dose.

6 Contribution

All authors contributed equally for this review paper.

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8 Competing interests

The authors declare that there is no competing interest.

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