

Biologically Active Triterpenoids and Their Cardioprotective and Anti-Inflammatory Effects

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Abstract

A diet rich in fruits and vegetables has been identified to confer multiple health benefits, including the reduction of cardiovascular disease risk. Fruits like apple, grape berry, olive, tomato, and mango all contain triterpenoid compounds with cardio-protective and antioxidant activities that can significantly attenuate or delay cardiovascular disease onset. Various clinical trials have been conducted on humans assessing the potential role of triterpenoid usage in the prevention of such chronic disorders, and the possible mechanisms responsible for the observed therapeutic actions. This paper is a review of primary research articles investigating the biologically active triterpenoids and their cardioprotective and anti-inflammatory effects.

Keywords: Triterpenoids; CVD; Inflammation; Ursolic; Oleanolic; Erythrodiol; Amyrin; Lupeol; Botulin; Nutraceutical

Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in Western countries [1]. CVD is a disease that causes the highest mortality rate globally [2], and is induced by risk factors such as inflammation, elevated serum total cholesterol, low-density lipoproteins (LDL), low serum high-density lipoproteins (HDL), diabetes mellitus, and advancing age [2]. Inflammation remains as a leading hallmark for an outcome of CVD, and is a body's physiological response to external or internal stimuli such as tissue injury and infection [3]. Inflammation is also one of the mechanisms that can cause heat stress to result in decreased muscle growth [3], which may trigger physiological dysfunctions of cardiac myocytes [4]. The resulting dysfunctions lead to mitochondrial apoptosis, the process of programmed cell death, which can later lead to CVD [4]. Fortunately, research suggests that the majority of CVD events may be prevented through controlled or modified dietary intervention.

Fruits and vegetables have been identified through epidemiological evidence to contain phenolic and triterpenoid (or triterpene) compounds with cardio-protective agents and antioxidants that can significantly attenuate or delay CVD onset [5]. Triterpenoid content appears to be positively correlated with reduced symptoms of chronic obstructive pulmonary disease and decreased risk of thrombotic stroke [6]. The health benefits of dietary triterpenoids, a group of plant secondary metabolites, are a more recent discovery; therefore there has been less research to date than on phenolic compounds. Research also suggests a positive association between reduced reported incidences of coronary heart disease with consumption of triterpenoids found in natural resinous materials and plants [7]. This paper will be a systematic review investigating the beneficial effects on inflammation and CVD of triterpenoids from different fruits: apples, grape berries, olives, tomatoes, and mangoes.

Triterpenoid Structure

In order to understand the association between triterpenoids and CVD, it is essential to study the protective action of a group of secondary metabolites produced by certain types of plants called pentacyclic triterpenoids. Pentacyclic triterpenoids, derived from the linear hydrocarbon squalene, demonstrate direct and indirect antioxidant and anti-inflammatory activities that can confer bioactive benefits in animals and humans [7,8]. The acidic function and hydroxyl (-OH) groups of the triterpenoids cannot interact with the stationary phase, as the two groups are located on the opposite sides of the compound [7].

There are three main triterpene families: oleane, ursane, and lupane triterpenes. The main triterpenoids found in the oleane family are oleanolic acid, erythrodiol, and β -amyrin; in the ursane family are ursolic acid and uvaol; and in the lupane family are lupeol, betulin, and betulinic acid (Figures 1-4) [7].

Due to the low-volatility of triterpenoids, a derivatization step prior to analysis from in-vitro studies was conducted to identify over 50,000 pentacyclic triterpenoids present in natural products included in the human diet [7]. The identification of triterpenoids is not easy, as the triterpenoids can have various isomer positions and be converted to other triterpenoids depending on the enzymes in the fruit. For example, a cytochrome P450-dependent oxidase (CYP716A12) was identified as a β -amyrin 28-oxidase through an in-vitro assay, which is able to modify β -amyrin to oleanolic acid [9]. CYP716A12, obtained by fulllength cDNAs, was characterized as a multifunctional enzyme with not only β -amyrin 28-oxidase, α -amyrin 28-oxidase and lupeol 28-oxidase activities, but also as a potential generator of ursolic acid and betulinic acid [9].

Main Sources of Triterpenoids

Skin of the fruits

The surface tissue of fruits is not only the first line of communication system for the surrounding biotic and abiotic environment, but a protective barrier against water loss, chemical or biological attack, mechanical injuries, and microbial infection

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Figure 1: Structures of oleane family triterpenoids present in fruit cuticular waxes of grape berry (**V. vinifera**) (oleanolic acid (1), oleanolic aldehyde (2), erythrodiol (3), β -sitosterol (4), β -sitosterol-3-O- β -D-glucoside (5), β -sitosterol-6'-linolenoyl-3-O- β -D-glucopyranoside (6)) [8].





[10]. Most of the compounds are found in the peel of the fruit, especially within the cuticle [7]. The cuticle tissues are epidermal tissues and subepidermal tissues that constitute the skin of the fruits lacking hard tissue [11]. They are lipidic layers mainly composed of biological insoluble polyester, called cutin (Figures 5a and 5b). Cutin is a polyester polymer rich in hydroxylated and epoxy-hydroxylated C16 and C18 fatty acids, found within an impermeable wax complex. The prominent components of the cuticular waxes are n-alkanes and triterpenoids [11].

Ursane triterpenes

C29 hydrocarbon n-nonacosane is one of the major n-alkanes compounds in the cuticle of apples [11]. The cutin of the apple membrane is found in the cuticle, and it constitutes over 50-60% of the weight of the overall apple. With the main monomer type being 18-OH-C18 [11], and with high epoxides content (35-40%) as well as over 50% of unsaturated components, the cutin of apple fruits are composed essentially of the following constituents: 18-hydroxyoctadeca-9,12dienoic, ursolic(10,16-dihydroxyhexadecanoic), 9,1-O-epoxy-18hydroxyoctadecanoic, 9,1-O-epoxy-l-8-hydroxyoctadec-12-enoic, and 9,10,18-tri hydroxyoctadecanoic acids [12]. As mentioned previously, ursolic acid (UA) is part of the ursane triterpene family. He et al. (2012) found that in certain apple fruits, UA was the main active constituent amongst the other triterpenoids, and the acid contents varied depending on the cultivar [6]. For example, in Malus pumila Mill, ursolic acid was the predominant compound composing over 98% of the triterpenoid mixture [8]. Research finds that in the human diet, apples (Malus pumila) are one of the greatest sources of phytochemicals and antioxidants [5], being rich in phenolic compounds [13]. However, there have been limited studies conducted which focused on the range of triterpene cultivars, its variations, and its growing or storage conditions in apples [14].

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Figure 4: Chemical structures of lupane family: (a) betulinic acid, (b) betulin and (c) lupeol [111,112].

Apple peel triterpenes

Apples compose a significant portion of the human diet, with a massive global production of approximately 62 million tonnes per year [15]. Earlier studies have explored the effectiveness of apple on its cardioprotective properties. Different tissue types individually containing phytochemicals in different compositions compose apple fruits, such as the peel, cortex, core, and seed. Many different compounds have been identified in the apple peel such as: organic acids, phenolic acids, flavonoids, coumaryl fatty acid esters, sesquiterpenes, and triterpene acids. Triterpene acids are documented to confer a number of potential health benefits [16]. Methods using the ultra-performance liquid chromatography-electrospray ionization MS analysis determined that fruit peels, pomace, flesh, and juice are a good source of high levels of bioactive triterpenes, with the peel being the richest source [6]. As well, triterpenoids isolated from apple peel had demonstrated more potent antioxidative and antiproliferative activity, when compared to the apple flesh [6].

The integrity of the apple peel significantly impacts the apple fruit harvest seasons as it can control water loss and level of protection from external pathogens. It can act as a barrier physically and chemically to deter insects [16]. Pentacyclic triterpenes are present mostly in the surfaces of plant to protect the plant from external and internal stress inducers [14]. The delineation of the climate did not mark any change in the level of triterpenoid acid compounds, suggesting that the triterpenes present in the peel are derived from biosynthesis completed at a preclimacteric stage. There were no marked changes in reserves of the compounds "on" or "off" the tree [16]. Playing a vital role in maintaining







Figure 5b: Labeled cutin deposition in a leaf. Epicuticular waxes cover the cutin embedded with intracuticular waxes, called cuticle proper. Additional layers include: cuticular layer; middle lamellae; primary cell wall; plasma membrane; cytoplasm; and vacuole.

the life of the fruit, apple peel composition has been extensively studied to understand its potent antioxidant capacity [16]. The peel of apples is comprised of the outer wax layer and the underlying layers composed of epidermal cells [16]. Studies conducted through radiotracer methods show that the site of cutin synthesis, forming over 30 constituent acids, is in the epidermis of the peel [12]. In the wax of the apple peels [9], ursolic acid ((3 β)-3-hydroxyurs-12-en-28-oic acid) and oleanoic acid (3 β -hydroxyolean-12-en-28-oic acid) derivatives with hydroxyl, oxo, and coumaroyloxy groups were found [16]. Through the measurement of differences between inflammation-related gene expression and markers and bacterial populations in mice and humans fed specific diets containing different apples, it was determined that apple peel extracts containing these triterpenoids could induce antioxidant concentrations in plasma and serum while reducing damage in DNA, inflammation, and oxidative stress levels [17].

A study conducted by Yang et al. (2014) discovered that treatment using ursolic acid has been found to have three important effects in mouse cardiac myocytes [4]: 1) attenuate myocardial apoptosis; 2) mediate anti-apoptotic and antioxidative activities against Endoplasmic Reticulum (ER) stress-associated myocardial damage; and 3) up regulate inactivate CHOP-induced Puma, a downstream and proapoptotic gene interconnected with over production of ER stress [4]. Another study conducted by Andre et al. (2012) using phenolic and triterpene contents among 109 apple cultivars exhibited the potential of triterpene-rich fraction of apple peel on reduction of the activity on the human tumor necrosis factor-alpha (TNF-a) promoter [14]. TNF-α gene polymorphism is linked to the causation of atherosclerosis [18]. The development of complications such as atherosclerosis and CVD appears to be stirred up by inflammatory cytokines, which prompts expression of the involved mediators [18]. Therefore, through the regulation of TNF- α gene, the development, progression, and complications of atherosclerosis secreted by inflammatory cells, and thus risk of CVD, are reduced [14]. Apple peels also exhibited lipaseinhibitory activity, which may offer important implication for the prevention of atherosclerosis and myocardial ischemia [6,19].

Many findings support the beneficial actions of apple peel on oxidative stress and inflammation: anti-hypertensive effects, inhibition of platelet aggregation, and increase in endothelial-dependent vasodilation [20]. Through the consumption of apples, the risks of various chronic diseases such as CVD appeared to have decreased in multiple researches. This appeared to have been through the mechanism of the fruit that lowers overall blood cholesterol level and inflammation [13]. Significantly reduced plasma cholesterol, LDL cholesterol (LDL-C) and triglyceride concentrations resulted on several animal and human apple consumption trials [21-24].

Ursolic acid also has the potential to reduce the adverse consequences of heat stress in mouse cardiomyocytes through the regulation of Mcl-1 pro-apoptotic protein, and restoration of the intracellular redox state [4]. With results from various studies that investigated the protective effects of ursolic acid (UA) on inflammation and atherosclerosis, it therefore could be concluded that UA can prevent inflammation and may have positive significance for prevention and treatment of atherosclerosis and other CVD diseases [25-27].

Oleane triterpenes

Oleanolic acid (OA), maslinic acid (MA), and β -amyrin are all part of the oleanane triterpene family. These compounds can be found in the peel of grapes, olives, and tomatoes, respectively. OAs are found to be isolated from over 1620 plant species, and they can be used for chemical

modification as they have the capacity to act as starter molecules. This can improve the natural bioavailability of grapes by producing more effective compounds through the modification on the C-3 hydroxyl, the C-12 C-13 double bond, and the C-28 carboxylic acid, thus contributing to the fruit's overall anti-oxidant, anti-inflammatory, and cardiovascular activities [7].

MA compounds were identified as the major components of the chloroform-soluble waxes of various types of olive fruits accounting for up to 68% of total wax extract [28]. In-vitro results demonstrate that maslinic acid compounds have previously exhibited antioxidant properties against lipid peroxidation. This suggests the possibility of using olives for the prevention and treatment of hyperlipidaemia, which is a risk factor for CVD [29]. Lastly, the amyrins could serve as structural elements of the cuticle and as the cuticular barrier [30]; with the ratios of 2:3:3 in MicroTom respectively, or 2:2:3 in most other cultivars, including M82 and Alisa Craig [30-32], amyrin-type pentacyclic triterpenoids are associated with the protection against photo-oxidative stress [30].

As these important groups of phytochemicals exert numerous biological effects and display various pharmacological activities that may be beneficial, this section will explore the triterpenoid contents of grape, olive, and mango fruits, and their effects on CVD and inflammation.

Grape skin triterpenes

Similar to apple fruits, grape products have recently gained substantial attention for their potential beneficial health effects [33]. Grapevine (*Vitis vinifera* L.) and its fruits have been valued highly for their economic importance and health benefits by various cultures throughout history [8]. For example, the phenomenon called the "French paradox" has been used to correlate decreased CVD incidences with the large consumption of wine in France.

Well-known for their antioxidant content [8], grape products have been extensively documented for their capacity in decreasing oxidative stress and inflammatory markers. The levels in urinary F2-isoprostanes, which is a whole-body oxidative stress marker, and the plasma tumor necrosis factor- α , which is an inflammatory cytokine [34] both were reduced in subsequence to grape product consumption. There was also a positive correlation between grape product consumption and the decrease in superoxide production [35,36]. Other suggested beneficial effects of grapes were the reduction of LDL-C and apolipoprotein B-100; induction of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I concentrations; and reduction of serum cholesterol levels and atheroma development. These alterations of levels in lipoproteins, serum cholesterol, and plasma lipids all were found to be protective mechanisms against atherosclerosis, which is a common cause of CVD [36-39].

Furthermore, several human and in-vitro studies have found that grape products may reduce CVD risk by lowering the low-density lipoprotein susceptibility to oxidation [36,37,41-43]. As triterpenoids are a group of secondary metabolites that may confer various beneficial biological activities, active investigation in the field of cardiovascular health recently began to focus on the antioxidant and anti-inflammatory effects of triterpenoid compounds found in grape products [44]. Triterpenoids present in grape cuticular waxes therefore became a growing topic of interest due to their role in protection against biotic stresses, their impact on the mechanical toughness of the fruit surface, and their potential industrial application [45]. A large amount of waxes compose the grape berry cuticle, forming around 25% of the overall cuticular weight [46,47]. Homologs of CYP716A12, CYP716A15, and CYP716A17 involved in triterpenoid biosynthesis were highly expressed in grape stem and skin, especially at the young and intermediate stages [9]. Various triterpenoids were identified in grape berry cuticular wax, including oleanolic aldehyde, β -sitosterol, β -sitosterol-3-O- β -D-glucoside, β -sitosterol-60-linolenoyl-3-O- β -D glucopyranoside, and erythrodiol [8,48-49]. Other identified common compounds include oleanolic and ursolic acid methyl esters, α -amyrin, α -amyrenone, β -amyrin, cycloartanol, 24- methylenecycloartanol, germanicol, campesterol, stigmasterol, and lupeol [9].

One of the several types of the triterpenoid compounds found in the peel is oleanolic acid (OA) [50]. In the fruits of the sultana vines and several American Vitis species, OA was the main constituent of the chloroform extracts of cuticular waxes of the fruits [8]. OA was also found to compose up to 86% of the triterpenoid mixture in grapes in a study by Zhang et al. (2004) [47]. In young and mature sultana vine fruits, the OA content ranged from 45 to 65%, respectively. This conflicted with the conclusion by Pensec et al. in that the oleanolic acid level is highest in young grapes in comparison to mature grapes [44]. In dried grapes, the acid constituted for 50% of the total wax extract [51].

When calculated for the whole fruit skins, the oleanolic acid content ranged from 157 up to 239 mg/kg of fresh berry mass in various grapes [52]. This abundance of oleanolic acid may be a main contributor to the many ascribed grape consumption health benefits. Similar to its isomer ursolic acid that is found abundantly in apple peels, oleanolic acid can also confer numerous pharmacological properties and have cardioprotective effects. For example, OA was previously found to inhibit COX-2 enzyme activity by 10% [53]; inhibition of COX-2 enzyme activity can reduce the risk of inflammation. Various recent studies have found the effect of OA to be beneficial to the heart; the study by Marquez-Martin et al. (2006) concluded that in human mononuclear cells, OA promotes an anti-inflammatory cytokine profile and interferes with the generation of cardiac-specific antibodies, thereby developing and establishing the prevention and treatment of experimental autoimmune myocarditis (EAM) [54-57]. As well, OA was found to be a novel helpful tool even when reactive or inflammatory cells have already begun to infiltrate the myocardium by suppressing myocarditis and conferring protective effects on cardiac cells and inflammatory cardiomyopathies [57]. Another study by Doronzo et al. (2013) concluded that OA increases vascular endothelial growth factor (VEGF) synthesis and secretion in rat vascular smooth muscle cells (VSMC) [58-71]. VEGF action on VSMC is essential for collateral vessel formation, and poor collateral vessel formation has been proposed to lead to the pathogenesis of obesity vascular complications [71]. Similarly, Mapanga et al. (2012) concluded that OA treatment in streptozotocin diabetic rats resulted in improved heart function and cardioprotection through the reduction of oxidative stress, HBP flux, and apoptosis in heart cells, thereby offering a potential novel therapeutic intervention to treat patients with associated cardiovascular complications [71].

As the derivatives of oleanolic acid became known to be relatively non-toxic and cytoprotective, Xing et al. (2012) also became interested in synthesizing a novel derivative of oleanolic acid, dh404, and its potential effects on lowering biomarkers for CVD [54]. The results of their studies on mice found that dhd404 increased myocardial levels of Nrf2 and Nrf2 nuclear translocation, with a dramatic oxidative stress suppression in the heart. Dh404 also acted as an inhibitor for hypertrophic growth and death in cardiomyocytes and suppressed the proliferation of cardiac fibroblasts [54]. Dh404 administration led to the inhibition of the pathological cardiac dysfunction, and thus reduced the mortality rate [54]. These findings indicated a therapeutic potential of OA for cardiac disease [54]. Bachhav et al. (2011) found that OA consumption had antioxidant and nitric oxide releasing action, which significantly decreased the level of plasma nitrate/nitrite [55]. Their study further suggested the potential prevention of hypertension and CVD risk through OA consumption [55,73].

Olive triterpenes

Vast interest has previously been devoted to characterizing the ways in which pentacyclic triterpenoids in olives mediate health and disease prevention. Various properties that may be useful in modulating cardioprotection activity, anti-inflammatory activity, and antioxidant protection have previously been identified upon the consumption of the olive fruit [56,73]. For instance, cell oxidative damage is related to multiple diseases that could be prevented through the antioxidant properties of olive oil triterpenes [56,57].

Triterpenic acids present in olive fruits are bioactive compounds that may exhibit multiple nutraceutical activities. The main triterpenes found in olives, olive tree leaves, and virgin olive oil are oleanolic acid, uvaol, erythrodiol, and maslinic acid [56,60]. The concentration of these triterpenes depends on the quality of the olive, the type of the tree, and the degree of ripeness [56,58].

Stiti et al. (2007) discovered that the triterpenic acid concentrations of olive fruits are dependent on the stages of the plant maturity [58]. During the development phase of the fruit between 12 and 18 weeks after flowering (WAF), the olive fruits were found to contain high concentrations of more-oxygenated compounds such as triterpenic diols (erythrodiol and uvaol) and acids (oleanolic, ursolic and maslinic acids) [58]. However, after the fruit matured and reached its final size between 21 and 30 WAF, triterpenic acids, essentially maslinic acid, replaced the triterpenic diols [58]. As well, when the concentration of the pentacyclic triterpenoids present in the Picual and Cornezuelo olive fruits were measured by HPLC-UV/vis, MA and OA appeared to be the only two pentacyclic triterpenoid compounds present, with MA being more abundant in content [59]. Maslinic acid (2a,3\beta-dihydroxy-12oleanen-28-oic acid, MA) is a found pentacyclic triterpene from Olea europaea L., that is particularly present in high concentrations in the olive pomace [60-62]. The olive pomace contains the stems, pulp, seeds, and peels of the fruit.

The skin of olives included large amounts of pentacyclic triterpene acids, namely maslinic and oleanolic acids; the cuticular waxes were the main location of the two compounds in various studies that investigated the triterpenoid composition of the olive skin [63-65].

Maslinic acid (MA) compounds were identified as the major components of the chloroform-soluble waxes of various types of olive fruits [66]. As mentioned previously, maslinic acid compounds have exhibited antioxidant properties against lipid peroxidation, which may suggest that olives have the potential to prevent and treat hyperlipidaemia, a risk factor for CVD [57]. For an instance, histologically, there was a found decrease in lipid accumulation and modulation of hyperlipidaemia-induced abnormalities upon the olive pomace extracts treatment [67]. MA not only exerts hypoglycemic effects, but studies have also found its antioxidant and cardioprotective effects with no harmful reaction as a nutraceutical [67-69]. Montilla et al. (2003) also concluded that MA may offer advantages in the oxidative stress resistance, through an animal-based study conducted to see the effects of MA on the susceptibility of plasma membranes or membranes of hepatocyte to lipid peroxidation [70].

Similarly, Guan et al. (2009) also suggested that MA is a potent inhibitor of excessive glycogenesis and is an inducer of cellular glycogen content, which could play a role in CVD risk reduction [71]. MA also reduced intracellular ROS level and prevented H2O2-induced oxidative injury in cultured cortical astrocytes [72]. Results showed that maslinic acid also dose-dependently normalized the caspase expression/ activation while increasing the Bcl-2/Bax ratio, to modulate apoptosis associated with progression of diseases [73-77]. The oxidation of the peroxidase enzymes in a study by Marquez-Martin et al. (2006) also indicated that pre-treatment with MA reduced hydrogen peroxide generation from stimulated macrophages in a dose-dependent manner in human mononuclear cells [58]. This hydroxyl-pentacyclic triterpene derivative therefore offers a biopharmaceutical potential on preventing generation of oxidative stress and pro-inflammatory cytokines; cytokines are released proteins with specific effects on the interactions, communications, or the behavior of cells [57].

In other studies, the potent anti-inflammatory effect of MA treatment was found to be exerted through the inhibition of the oxygenglucose deprivation-induced production of Nitric Oxide (NO) and TNF-a [75]. There was a significant suppression of the expression of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) at protein and mRNA levels [75]. Moreover, analysis through Western blot technique found that MA attenuated LPS-induced translocation of NF-кB p65 subunit to the nucleus. NFкB is an important transcription factor expressed in all mammalian cell types [75]. In arteries, NFkB is believed to promote CVD through its pro-inflammatory, pro-adhesion and pro-oxidant gene transcription [75]. Even in patients already suffering from symptoms of CVD, systemic administration of MA may be beneficial in the alleviation of inflicted pain caused by the disease, as it has been previously found that topical administration of MA reduced acetic acid-induced writhing, as well as the inflammatory phase of formalin-induced pain and capsaicin-induced mechanical allodynia [76].

Tomato triterpenoids

Over the last century, tomato (*Solanum lycopersicum* L. or *Lycopersicon esculentum* L.) has gained great popularity by the general public and the scientific community for their potential health benefits [8]. Being cultivated globally both indoors and outdoors [8], it is a fruit that has been studied extensively.

In terms of their triterpenoid content, research has especially focused on the cuticular waxes of the tomato fruit more than most other fruits [8]. The main cutin monomers of the tomato fruit cuticle include 16-hydroxyhexadecanoic acid, 10,16-dihydroxyhexadecanoic acid, and 18-hydroxyoctadecanoic acid [30]. The fruit surface and intracuticular waxes are predominately composed of very long chain alkanes, fatty acids, C_{32} -n-aldehyde, and triterpenoids [31,76-78]. The most common types of triterpenoids in the tomato fruits are the amyrins (α -, β -, and δ -amyrin), accounting for approximately 76% to 91% of the overall cyclic wax constituents [79,80].

Depolymerization by BF3/methanol for cutin analysis conducted with the extracts from the cutin matrix and the cuticular waxes examined the cuticular composition during the tomato fruit development [30] to find that approximately 25% of the total triterpenoids of the whole fruit is found on the surface wax in mature MicroTom tomato fruits [31].

It was revealed through parallel metabolite analysis that 74%,

79%, and 74% of the total α -amyrin, β -amyrin, and δ -amyrin are present in the surface wax, respectively, while the remaining 21% to 26% are found in the underlying epidermal cell layer and the internal tissues of the tomato fruit [80]. Analysis was conducted for the gene expression and metabolism in tomato fruit surface tissues at five stages of fruit development to discover that the level of the three triterpenoids increased significantly at the mature stage [30]. More specifically, α - and β -amyrins (AMY) are bioactive compounds that have been identified as anti-inflammatory and anti-oxidant agent agents by multiple sources and thus used for the treatment of diverse inflammation-related diseases [80-104]. The chemical formula of α -amyrin(3 α -hydroxy-urs-12-en-3-ol) is C₃₀H₅₀O (Figure 2), and its melting point is 184-186°C [103]; the chemical formula of β -amyrin(3 β -hydroxy-olean-12-en-3ol) is C₃₀H₅₀O (Figure 2) [106-112] and its melting point is 189-191°C [84].

Quintão et al. (2014) demonstrated that AMY interferes in both acute and chronic inflammatory processes in mice by reducing mechanical hypersensitivity, mechanical sensitization, and oedema development in arthritis [86]. In a study by Krishnan et al. (2014), significant decrease in edema was observed in rats by the administration of β -amyrin in a dose-dependent manner. Thus, β -amyrin serves as a promising and expanding platform for treatment of various inflammatory disorders [87]. Other studies, for example by Melo et al. (2010), also found that α - and β -amyrins have beneficial effects in decreasing the serum levels of amylase and lipase, which therefore supported that AMY acted as anti-inflammatory and antioxidant agents [82].

Therefore, from the conferred results of various studies investigating the beneficial effects of amyrins, the suggestion of triterpenoids being potential new therapeutic methods of painful and inflammatory diseases management, hence CVD, seems reasonable, especially in those presenting a chronic profile [85].

Mango lupane triterpenes

Many triterpenoids are known for exhibiting similar significant pharmacological properties; lupeol for example is known to have properties that significantly influence anti-inflammatory activities [86]. Lupeol (Lupa-21, 20(29) dien 3 beta-ol) is a naturally occurring pentacyclic triterpene (also known as Fagarsterol) under the lupane triterpene family, predominantly present in mango [87,88]. As there has been limited number of studies conducted that focused on the effect of lupane triterpene compounds on health, this section will focus on the triterpenoid composition of mango fruits and their effects on CVD and inflammation [14].

Mango (*Mangifera indica* L.) is a fruit consumed worldwide [91], being one of the most commonly consumed fruits in the tropical countries [93]. Having been part of the indigenous medical systems for over 4000 years, mango fruits have been considered a good source of bioactive compounds that can be used towards preventing diseases and promoting overall good health in humans [90].

As life style and dietary habits can be considered major determinants of the causation or prevention of genetic disorders [91], epidemiological evidences have demonstrated the efficacy of mango fruits consumption in disease prevention. Diverse compounds with bioactive properties were identified in mangoes, attracting the attention of consumers in the general public and the scientific community [90]. Research within the last 20 years has revealed that even the mango byproducts from industries could potentially serve as a valuable source of bioactive compounds [89]. Bioactive compounds are very important in the food and pharmaceutical markets, and there is a growing interest in conducting researches on developing more efficient methods for extracting the bioactive substances in mangoes, mainly triterpenoids [90].

Various studies have identified mango to possess various properties in both vivo and in-vitro studies: anti-oxidant, cardiotonic, antihyperlipidemic (antiatherosclerotic), and anti-inflammatory [88,92,93]. High concentrations of lupeol are found in the peels of mango fruits, especially during the maturity stage [90]. In animal studies, the oral administration of lupeol improved the antioxidant status of the liver and scavenged the free radicals to alter the tissue redox system [90]. In vitro, lupeol was also found to significantly reduce the free radical mediated DNA-sugar damage and microsomal lipid peroxidation [88].

As reactive oxygen species (ROS) possess a strong oxidizing effect and induce damage to biological molecules by changing the structure and function of proteins, lipids, and DNA [88], lupeol is found to confer significant antioxidant effects by decreasing ROS levels with restoration in the levels of lipid peroxidation and antioxidant enzymes [88,91,92]. These antioxidant effects included free radical scavenging properties that led to the induction of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activities, which are found to act against isoproterenol in the heart [96]. For these beneficial effects with no toxicity in normal cells and tissues, tremendous efforts by researchers were made globally to develop lupeol for its clinical use within the last 15 years [95].

In a variety of cells, the key molecular pathways considered prototypical pro-inflammatory signaling pathways were targeted by lupeol: nuclear factor kappa B (NFkappaB), cFLIP, Fas, Kras, and Wnt/ beta-catenin [95,96]. Phosphatidylinositol-3-kinase (PI3K)/Akt, an anti-inflammatory signaling pathway, and 12-O-tetradecanoylphorbol-13-acetate (TPA) were targeted to mediate nitric oxide synthase and have effects on acute and chronic inflammatory processes [100-102].

Lupeol linoleate, an ester derivative of lupeol obtained through linoleic acid esterification, exhibited more potent anti-inflammatory and cytoprotective effects than lupeol [102,103]. As cellular cholesterol homeostasis is crucial for CVD prevention, it was important for Sudharar et al. (2007) to investigate the effects of both lupeol and its ester lupeol linoleate on the lipid status and biochemical changes on the heart tissues on male albino Wistar rats [106]. Sudharar et al. (2008) found that there were reductions in the total levels of cholesterol, triglycerides, and phospholipids upon the consumption of lupeol and its derivatives in the myocardium [104]. As well, the treatment decreased the activities of cardiac enzymes—lactate dehydrogenase (LDH), aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase—to normalcy [103], while it prevented the hypertrophic cardiac histology and restored the normal ultrastructural architecture of the heart tissue and muscle cells [104].

As hyperlipidemia and hypercholesterolemia are major risk factors for coronary heart disease and atherosclerosis development, the induced minimization of lipid abnormalities and abnormal biochemical changes through lupeol and lupeol linoleate consumption further suggests the cardioprotective effects of the triterpenoids [104]. Other agents that induce fatal cardiotoxicity, such as cyclophosphamide (CP), were also inhibited through the cardioprotective properties of lupeol and lupeol linoleate [104].

These observations that highlight the antioxidant property of triterpenes and their protective actions against induced cardiotoxocity

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further support the aim of this paper which investigates triterpenoids and their effects on CVD and inflammation.

Mechanisms of Triterpenoid Biological Activities

As various studies have reported a positive correlation between triterpenoid consumption and decreased CVD risk, this review examined the current scientific literatures concerning claims of cardiovascular benefits conferred from the consumption of triterpenoids, and the possible mechanisms responsible for such therapeutic actions. Evidence from multiple studies suggests that triterpenoid compounds work through various mechanisms to achieve favorable effects on CVD. A number of trials have been conducted to find out the usefulness of triterpenoid rich products against hypertension.

Triterpenoid compounds found in apple peel, mainly ursolic acid, were reported through a trial using unilateral ureteral obstruction rats by Lee et al. (2014); the triterpenoid compounds were reported to have anti-hypertensive effects, with capacity to inhibit platelet aggregation, and increase endothelial-dependent vasodilation [20]. Boyer and Liu (2004) conducted several animal and human trials to demonstrate that the usage of triterpenoids from apples utilize mechanisms that significantly lowers the overall plasma cholesterol level, LDL-C, and TG concentrations [13]; other studies concluded that the inhibitory mechanisms of TNF-a gene on lipase are regulated through consumption of triterpenoid compounds from fruits such as apples, grapes, and olives, which can prevent myocardial ischemia and the development of atherosclerosis [14,33]. By reducing the risk of atherosclerosis, cardiovascular disease risk may be reduced [99]. Apple peel triterpenoids also used antioxidant mechanism in plasma and serum to reduce the damage in DNA, inflammation, and oxidative stress levels [17]. Ursolic acid activated mechanism regulating Mcl-1 pro-apoptotic protein to reduce adverse consequences of heat stress in cardiomyocytes [4] and mediated anti-apoptotic and antioxidative activities against endoplasmic reticulum (ER) stress-associated myocardial damage.

Grape products similarly had triterpenoid compounds, mainly OA, that activated mechanisms that reduced oxidative stress, HBP flux, and apoptosis through increased myocardial level of Nrf2 and Nrf2 nuclear translocation [53,54]. OA also mediated the urinary F2-isoprostanes regulating mechanisms, superoxide production mechanisms, serum cholesterol level reducing mechanisms, and mechanisms that decreased the low-density lipoprotein susceptibility to oxidation; these mechanisms all conferred beneficial effects in CVD prevention [34-42]. OA, having anti-inflammatory cytokine profile that promotes mechanism that can interfere with the generation of cardiac-specific antibodies, developed prevention and treatment of EAM by conferring protective effects on cardiac cells and inflammatory cardiomyopathies [57]. OA also increases plasma atrial natriuretic peptide (ANP) levels, which is a mechanism involved with the regulation of cardiovascular homeostasis [110,111]. A novel derivative of OA is dh404, which functions with pathological cardiac dysfunction inhibition mechanisms and thus reduces mortality rate caused by death in cardiomyocytes and cardiac fibroblasts proliferation [54]. OA was also found by Bachhav et al. (2011) to confer nitric oxide releasing mechanism, significantly decreasing the level of plasma nitrate/nitrite [55]. In various studies, OA was found to inhibit COX-2 enzyme and Th1 responses, which can reduce the risk of inflammation [48,109].

Similar effects were found in maslinic acid (MA) products such as olive fruits, with a mechanism that significantly suppressed the expression of COX-2 and iNOS, in turn having an anti-inflammatory effect [75]. MA was also found to affect the mechanism that attenuate LPS-induced translocation of NF-KB p65 subunit to the nucleus [75]. NFkB is an important transcription factor believed to promote CVD through its pro-inflammatory, pro-adhesion and pro-oxidant gene transcription [75]. MA also offers advantages in oxidative stress resistance, as it targets mechanisms that reduce hydrogen peroxide generation from macrophages and intracellular reactive oxygen species (ROS) level, while offering no harmful reaction as a nutraceutical [67]. ROS possess a strong oxidizing effect, inducing damage to biological molecules; they are pivotal factors to the genesis of heart disease, as elevated level of ROS may cause an increase in oxidative stress that can initiate subcellular changes that can lead to cardiomyopathy and heart failure [97]. Results showed that MA prevented H₂O₂-induced oxidative injury and normalized caspase expression/activation while increasing the Bcl-2/Bax ratio to modulate apoptosis associated with disease progression [75].

Other triterpenoids such as α -and β -amyrins (AMY) as found in tomatoes had similar mechanisms in interfering with the acute and chronic inflammatory processes. AMY reduced mechanical hypersensitivity, mechanical sensitization and oedema, while demonstrating anti-oxidant, cardiotonic, antihyperlipidemic, and antiinflammatory effects [87]. Lastly, Sudharar et al. (2007) demonstrated that consumption of lupeol and its derivatives can reduce the total cholesterol, triglyceride, and phospholipid level, which can help regulate the overall myocardium mechanisms [106]. Cellular cholesterol homeostasis is crucial for CVD prevention, and these mechanisms were found to be effective in preventing the hypertrophic cardiac histology to restore the normal ultrastructural architecture of the heart tissue and muscle cells [104]. Lupeol also targets the ROS level decreasing mechanism through the levels of lipid peroxidation and antioxidant enzymes restoration, which induces CAT and SOD activities that act against isoproterenol in the heart [90]. As well, lupeol targets the anti-inflammatory signaling pathway, PI3K/Akt and TPA, to mediate nitric oxide synthase [94]. Pro-inflammatory signaling pathways and other fatal cardiotoxicity-inducing agents such as CP were inhibited by lupeol, which further supports the cardio protective effects of the triterpenoids [95].

Conclusion

From several in-vivo, in-vitro, and human studies, it has been demonstrated that many triterpenoid compounds present in fruits and vegetables possess potent and desirable biological activities that can protect against cardiovascular disease and inflammation, as summarized in Table 1. There are several mechanisms that may account for the antioxidant and anti-inflammatory biological properties of triterpenoids. The most universal properties related to their functions as antioxidants are manifested by their ability to trap free radicals; inhibit their enzymatic generation; and to block the oxidation of cellular and extracellular compounds. Even though this review has not investigated the maximum tolerated dose of triterpenoids, previous clinical studies have shown that the treatment of certain triterpenoids, such as maslinic acid, currently have no known side effects. The available evidence indicates that triterpenoid products could be helpful as medical treatment coadjuvants in specific situations, for patients under specific conditions. New studies should be aimed at improving the bioavailability of triterpenoids and discovering new analogues to help in finding more potent yet protective compounds in preventing diseases such as CVD.

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Oleane	Ursane	Lupane
 Antioxidant Antiproliferative Attenuation of myocardial apoptosis Mediation of anti-apoptotic and antioxidative activities against myocardial damage Inactivation of the CHOP-induced Puma up-regulation Reduction of the Tumor necrosis factor- alpha (TNF-α) promoter activity Reduced risk of atherosclerosis Reduced risk of CVD Beneficial effects on oxidative stress and inflammation Prevention of myocardial ischemia Reduced blood cholesterol levels Reduced blood cholesterol levels Reduced blood cholesterol levels Reduced blood cholesterol levels 	 Reduced levels of urinary F2-isoprostanes Reduced levels of plasma TNF- α Decrease in superoxide production Reduction of LDL and apolipoprotein B-100 Induction of HDL and apolipoprotein A-I concentrations Reduced serum cholesterol levels and atheroma development Reduced risk of CVD Antioxidant Anti-inflammatory Protection against biotic stress Inhibition of Th1 responses Suppression of myocarditis and conferring protective effects on cardiomyopathies Increase in ANP levels Increase of NO Inhibition of cardiovascular homeostasis Release of NO Inhibition of cellular glycogen context Reduction of intracellular ROS level and prevention of H2O2-induced oxidative injury Normalization of caspase expression/activation Increase of Bcl-2/Bax ratio to modulate apoptosis Attenuation of LPS-induced translocation of NF-xB p65 subunit to the nucleus Reduction of acetic acid-induced writhing and alleviation of formalin-induced pain 	 Antioxidant Cardiotonic Anti-inflammatory Alteration of the tissue redox system Decrease of ROS levels Induction of CAT and SOD activities Mediation of NO synthase through targeting (PI3K)/Akt and TPA Reduction of total cholesterol, triglycerides, and phospholipid levels

Table 1: Summary of the effects of fruit triterpenes on biological responses.

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