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Comprehensive overview: the effect of using different solvents for barley extraction with its anti-inflammatory and anti-oxidant activity

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Abstract

Barley (*Hordeum vulgare* L.) is one of the world's oldest cereal crops. There is considerable interest in barley's potential usage in human diets. Barley is rich in bioactive metabolites such as high content of β-glucan, fiber, and vitamin E. It is also well known as a rich source of phytochemical derivatives, namely, phenolic acids, flavonols, chalcones, flavones, proanthocyanidins, and flavanones. Phenolic compounds are recognized as excellent dietary materials with antioxidant and anti-inflammatory activities. This review was written to give an overview of the main components that are separated from barley using different solvents. Even though there were numerous biological activities for barely, the antioxidant, as well as the anti-inflammatory, are the main focus of this review.

Keywords

Barley. β-glucan. Bioactive metabolites. Cereal crops. *Hordeum vulgare.*

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1. Introduction

Cereals were related to food and drinks during the history of mankind, they acted as a major source of natural energy supply. It has a great benefit for human health. [1] Barley is one of the oldest cereal crops and is still in use nowadays. Barley was used by the ancient Egyptian; 10,500 years ago, along the River Nile. [2] According to archaeological findings, it ranks fourth among cereal grains after wheat, rice, and maize as one of the most widely farmed crops of both production quantity and cultivated area among grain crops in the world (12% of total cultivated cereals), [3] It contributed significantly to the advancement of human civilization, agronomic, physiological, genetic, and plant breeding sciences, which are grown and utilized all over the world. [2] Highland barley (HB, *Hordeum vulgare* L. var. nudum hook. f) is classified as a member of the Gramineae (wheat family). It is also known as hull-less barley or naked barley ("Qingke" in Chinese). This nomenclature is given due to the removal of the inner and outer glumes from the caryopsis when harvested, [4] Barley can be recognized in different forms and shapes; it could be found as two or six rows of seeds on each spike. It can also be hulled or hulless (based on the presence or absence of a tightly adhering hull to the grain). Barley can also be categorized based on grain content into normal, waxy (high amylose starch), high-glucan, and proanthocyanidin- free types. [1] Free, soluble conjugated, and insoluble forms of barley phenolic compounds exist, which are bound to the grain's cell wall components by ester or ether bonds and require acid, alkaline, or enzymatic hydrolysis to be released. [5] Barley has the highest levels of β-glucan compared to other cereals, followed by oat, rye, and wheat. [3] Barley has gained popularity as a commodity for the creation of functional foods due to its high level of physiologically active constituents. [6]

The nature and amount of secondary metabolites recovered from medicinal plants have been observed to be influenced by the type of solvents used during the extraction process, [7]

phenolic compounds' solubility in different polarity solvents is also determined by structural differences. As a result, solvent extraction and separation techniques may have a substantial impact on the yield of the phytochemicals extracted from the plant material. [8] Methanol, ethanol, acetone, and ethyl acetate have all been employed to extract phenolic contents from plant material. [8]

The goal of this work is to provide a thorough overview of several strategies for extracting bioactive chemicals from barley and shed light on the relationship between biological activities and the solvents used for extraction.

2. Traditional uses of barley

Almost 70% of the active compounds discovered in medicine come from plants, whereas only 30% are completely synthetic, [9] This grain is rich in soluble dietary fiber, particularly beta-glucans, and provides vital vitamins and minerals. [2] Idehen (2020) reports that barley may be beneficial as an antioxidant, anti-inflammatory, anti-diabetes, immunomodulation, antibacterial, cardiovascular disease and blood pressure control, gastroprotection, antiobesity, and antiaging. [10] Traditional healers utilize barley to treat a variety of inflammatory and cardiovascular disorders without understanding its pharmacological mechanisms. [11] Most of these activities are related to the presence of β-glucan, arabinoxylan, and polyphenols. [4] β-Glucans are major soluble fiber polysaccharides that have a great role in lowering plasma cholesterol, lowering blood glucose level, improving lipid metabolism, and reducing glycemic index. [10] Previous reports mentioned that regular consumption of barley has been linked to a lower risk of several ailments. There have been numerous scientific studies on the health advantages of green barley, including cancer prevention, hyperlipidemia, cardiovascular

disease, and other chronic disorders in addition to it is a good source of vitamins and minerals and has a lot of antioxidant activity. [2]

3. Anti-inflammatory effect

Inflammation is a complicated immunological response to damaging stimuli such as infections, damaged cells, and/or irritants, and it is linked to chronic disease progression. [12] Inflammation can also occur as a result of tissue damage, cell death, malignancy, ischemia, and degeneration. [13] During various forms of inflammatory responses, a variety of inflammatory mediators are synthesized and released. Pro- and anti-inflammatory mediators are the two main groups of inflammatory substances. [14] Various inflammatory mediators, including nitric oxide (NO), prostaglandins (PGs), and proinflammatory cytokines, activate macrophages, and active macrophages also create these mediators. Macrophages, in particular, play a key role in the production of interferon-g (IFN-g), interleukin (IL)-1b, IL-6, and tumor necrosis factor-α (TNF-α), which are all essential inflammation mediators. [12] Anti-inflammatory substances can be a helpful tool in the treatment of disorders.

4. Solvents used in the extraction of barley

4.1. Water extract

Water is thought to be a good solvent due to its safety. It is considered the most polar solvent. [15] According to the rule, like dissolves like, it was found that water extracts usually contain polar compounds. Shah, A., *et al.* used water as a solvent to isolate the polysaccharides β-glucan compound from the Indian barley. In several studies from different localities, such as Spain, India, and China, β-glucan was isolated and tested for its potential as an antioxidant using different techniques. These studies identified a wide range of biological activities, among them, wound

healing as well as, anti-inflammatory activities, and were investigated. [16, 17] One study tried to compare the antioxidant activity of the β-glucan obtained from microwaved hulled barley and an unprocessed one. This study showed that microwaved barley had greater activity. This result was related to the breaking of the polysaccharides chain as heating caused the exposure of more hydroxyl groups, increasing free radical scavenging activity. [18]

Gallic acid, protocatechuic acid, catechin, and caffeic acid were found to be the major compounds identified from various barley cultivars using the HPLC method. [19] Using the LC-MS technique, water extract from various Tunisian barley cultivars revealed a high content of *p*-coumaric acid and syringic acid and identified 19 compounds. [5] Different cultivars of juvenile barley (cereal sprout); plant raw materials showed that all water extracts are qualitatively similar, but differ quantitatively and catechin, epicatechin, quercetin, rutin, and kaempferitrin were the major identified compounds. A cholinesterase inhibitory effect was also recorded. [20] vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, and ellagic acid were identified from the water extract of barley purchased from the Egyptian market. [21] The water extract of young barley leaves from Korea was analyzed for its monosaccharide contents with the investigation of the immunostimulatory effect. [22] The antioxidant activity was tested in all those reports; all barley water extracts in different forms and from different locations showed great activity.

Some studies focused just on the biological activity of the water extract without a great deal of phytochemical analysis. The anti-depressant activity of barley leaves was examined [23] and another study dealt with hypolipidemic activities. [22]

As water can mainly extract polar compounds, most studies are concerned with an antioxidant activity using different mechanisms and techniques. The water extract of six varieties of spring barley from Mendel University in Brno showed antioxidant activity in the 2,2-Azino-bis(3-

ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTs) model in the range of 1.6–3.0 µmol/g, while DPPH model showed a range of 0.9 to 2.0 µmol/g. [24] The FRAP assay was used also to examine the antioxidant properties of water extracts of 19 Iranian cultivars of barley. [25] Barley seeds from the USA market were extracted by water and then tested for their antioxidant activity. [26] Water extraction of young barley powder was used to determine its anti-oxidant and antiproliferation activities. [27] Sumi Oh, BoRa Yi, *et al* (2014) revealed that roasting temperature has an impact on the antioxidant activity of the aqueous Korean barley by using different assays including ABTs, DPPH, and FRAP. [28]

Ruiz-Medina (2019) applied extracted green barley leaves with water to determine their antioxidant content by evaluating their phenolic content. [29]

The water and alkaline extracts of different huskless barley from China were tested for their anti-inflammatory activity, two of them blocked the overexpression of numerous important proteins in the human umbilical vein endothelial cells, including MCP-1, VCAM-1, and ACE, reducing the deleterious effects of TNF-α. [30]

The activities as well as the isolated compounds from barley were summarized in table (1) and figure (1) shows the major structures identified from the water extract.

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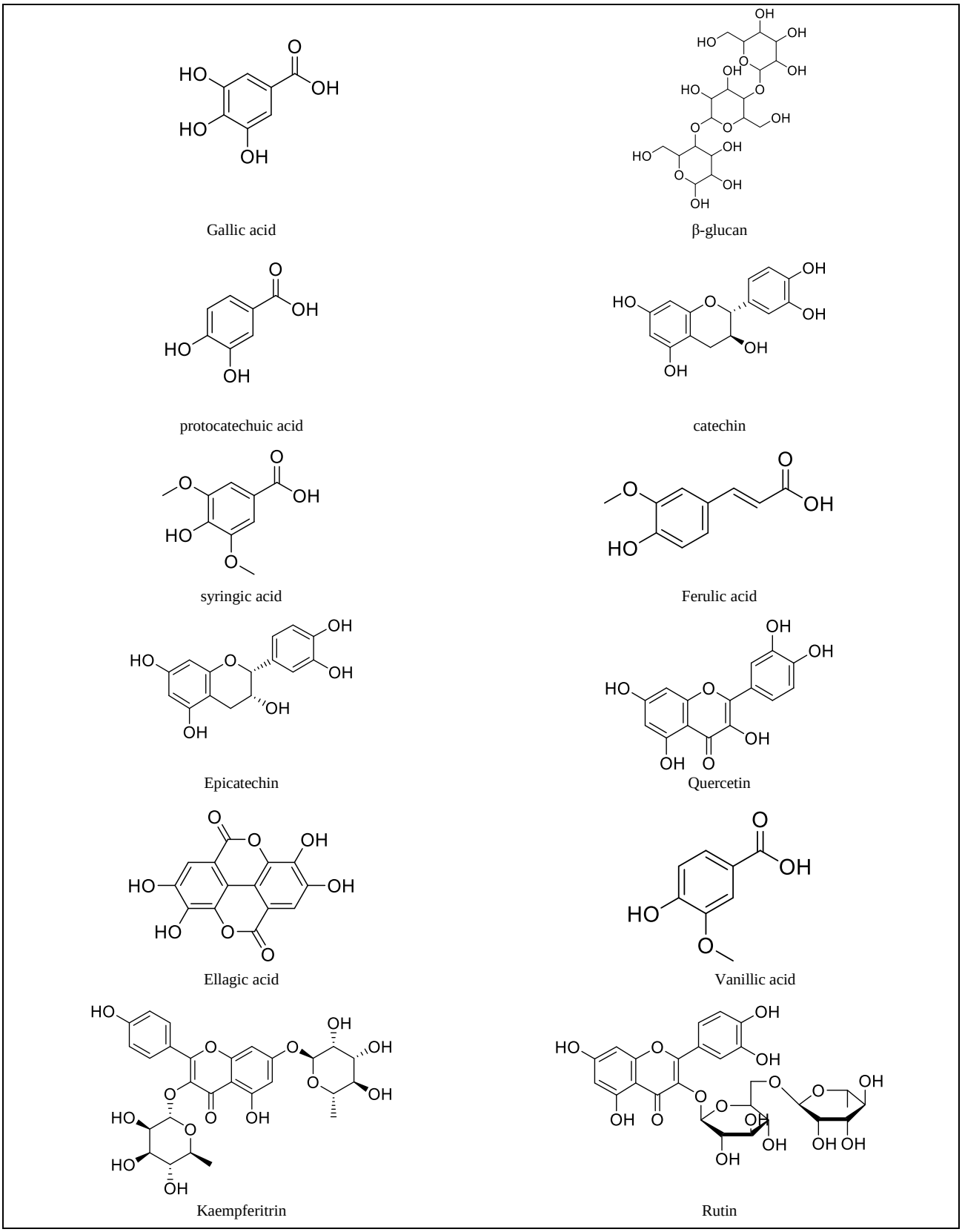

134 Fig. (1): Structures of the major compounds Identified in water Extract.

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4.2. Methanol extract

The biological activity of alcoholic extracts is generally more potent than that of aqueous extracts, mostly due to their high extraction power. The activity in most cases is related to the presence of higher levels of polyphenols, which are released from cells when organic solvents are used to break down cell walls, which have a nonpolar nature and cause polyphenols to be released. [31] The enzyme polyphenol oxidase, which degrades polyphenols in water extracts, is another more relevant explanation for the reduction of biological activity in the aqueous extract. [32] Usually, the combination of organic solvent and water facilitates the extraction of all compounds that are soluble in both solvents. Most studies used 70-80% methanol water as a solvent for extraction. Methanol extracts of barley from different locations result in the identification of proanthocyanidins such as catechin, catechin dihexoside, procyanidin B, procyanidin C, prodelphinidin B, and prodelphinidin C. [33, 34] Furthermore many anthocyanins were identified from naked (hulless) barley such as cyanidin-3-*O*-Glucoside, cyanidin-3-*O*-Rutinoside, cyanidin-3-Galactoside, cyanidin-3-*O*-(3″-*O*-malonyl-glucoside), cyanidin-3-*O*-(6″-*O*-malonyl-glucoside), cyanidin 3-*O*-dimalonylglucoside, pelargonidin-3-*O*-dimalonylglucoside and peonidin 3-*O*-dimalonylglucoside. [35, 36] Many flavone glycosides were identified from barley leaves methanol extract, such as apigenin, luteolin, chrysoeriol, and tricin glycosides. [37] 4-methoxy-5, 7-dihydroxy isoflavone (Biochanin A) was also identified in Pakistanian barley, besides the existence of alpha-tocopherol (Vitamin E). [38] Myricetin, quercetin & kaempferol were also identified from the barley sprouts in addition to many phenolic acids such as gallic acid, p-hydroxybenzoic acid, protocatechuic acid, vanillic acid. [33] Ge, X., *et al* (2021) extracted different colored naked barley grains with methanol. This resulted in the identification of seven phenolic acids and 15 flavonoids. Ferulic acid was the main phenolic acid in white naked barley, while vitexin was the highest of the

flavonoids. [35] Choi, Hwang, *et al.* (2013) identified several compounds from Korean barley where benzene propanoic acid 4-hydroxy-3-methoxy was the most abundant phenolic compound and hexadecanoic acid methyl ester was the major fatty acid. [12] Lee, J.H., *et al.* (2016) investigated seedlings of various Korean barley cultivars for the changes in phenolic acids and antioxidant capacities at four different harvest times. UPLC-PDA-ESI/MS revealed that lutonarin, saponarin, and isovitexin-7-O-glucoside) were the predominant phenolic (flavonoid) compounds in all tested samples. [39] Also, Seo, Park, *et al* (2014) isolated saponarin from barley sprouts and showed that saponarin reduces pro-inflammatory responses. [40]

The main class of compounds identified from the methanol extracts of different barleys is phenolic compounds (proanthocyanidin, flavonoid, and phenolic acid), which are well-known as excellent dietary materials with antioxidant, antiradical, and anti-inflammatory activity, as shown in all previous reports. [39] Gul, Ahmed, *et al*. (2014) showed that barley methanolic extract had an anti-inflammatory effect by determining glutathione peroxidase activity and superoxide dismutase activity[11]. Besides Choi, Hwang *et al*. (2013) determined the anti-inflammatory effect of barley methanolic extract using *in vitro* and *in vivo* assays. [12] This was demonstrated and reported by Woo, S.-Y., *et al*. (2021), which showed the efficacy of lutonarin and saponarin as anti-inflammatory. [41] Azelaic acid which has a strong anti-inflammatory effect was also identified as one of the main compounds in barely after planting for two weeks. [42] The antioxidant activity of barley extracts was tested using different assays such as DPPH, FRAP, and ORAC and it was found that the methanol extract had the highest antioxidant activity, [33, 36, 38, 43-46] The methanol extract of barley showed anti-bacterial activity against different pathogens. [47] Table (1) summarizes the activity, as well as the isolated chemicals, and figure (2), showed the major structures of identified compounds from the methanol extract.

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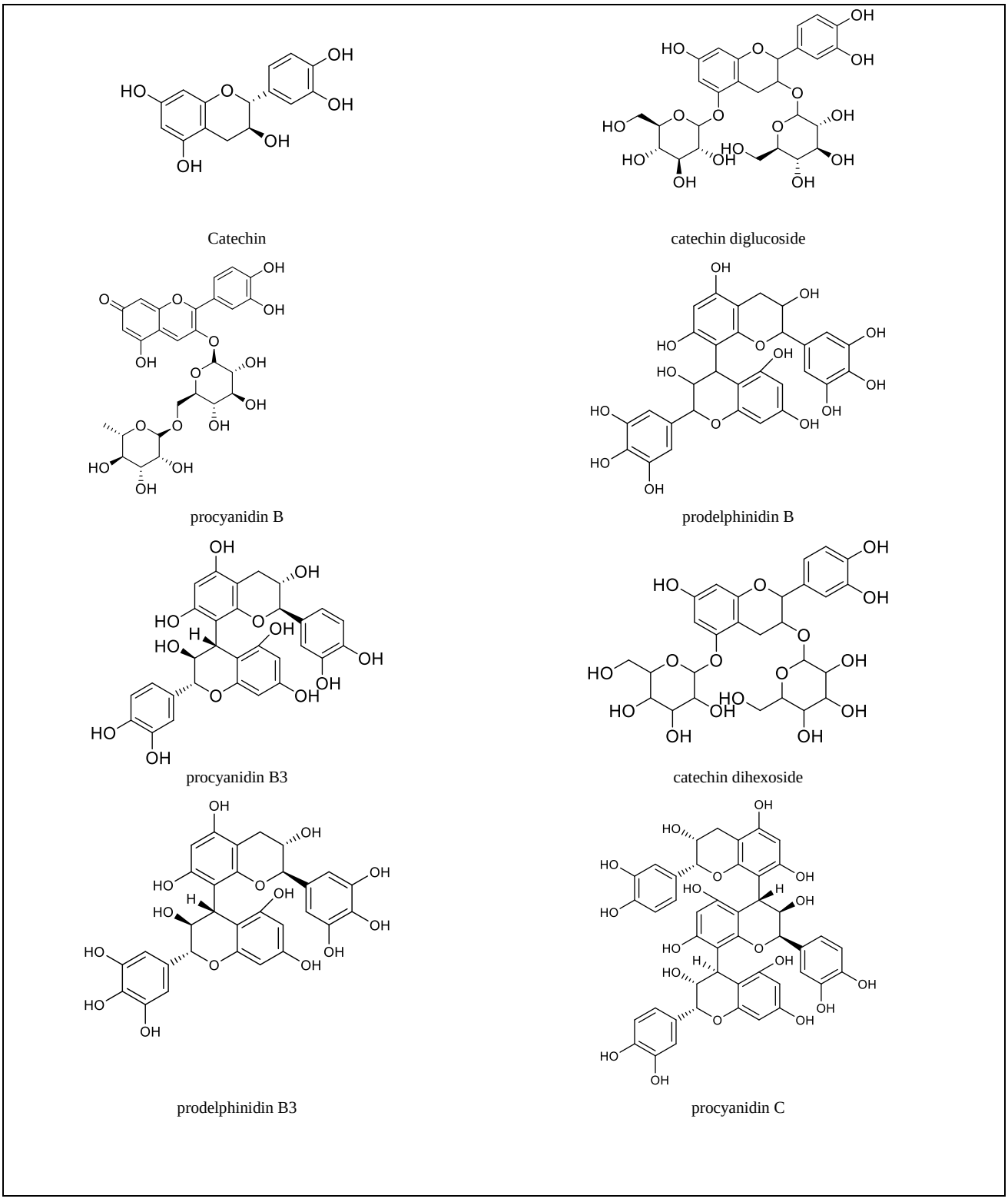

Fig. (2): Structures of the major compounds Identified in Methanol Extract

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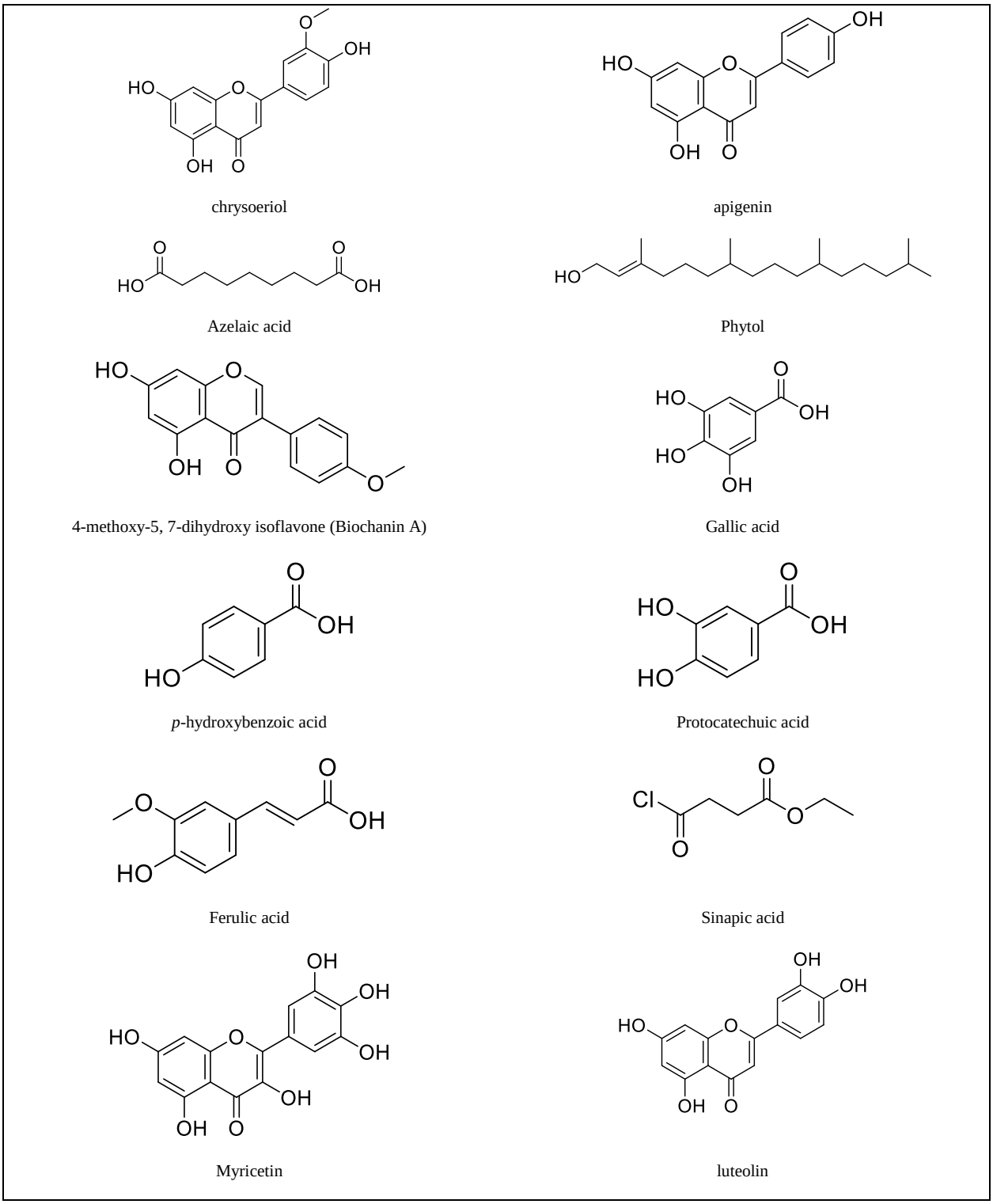

182 Fig. (2): Cont. Structures of the major compounds identified in Methanol Extract

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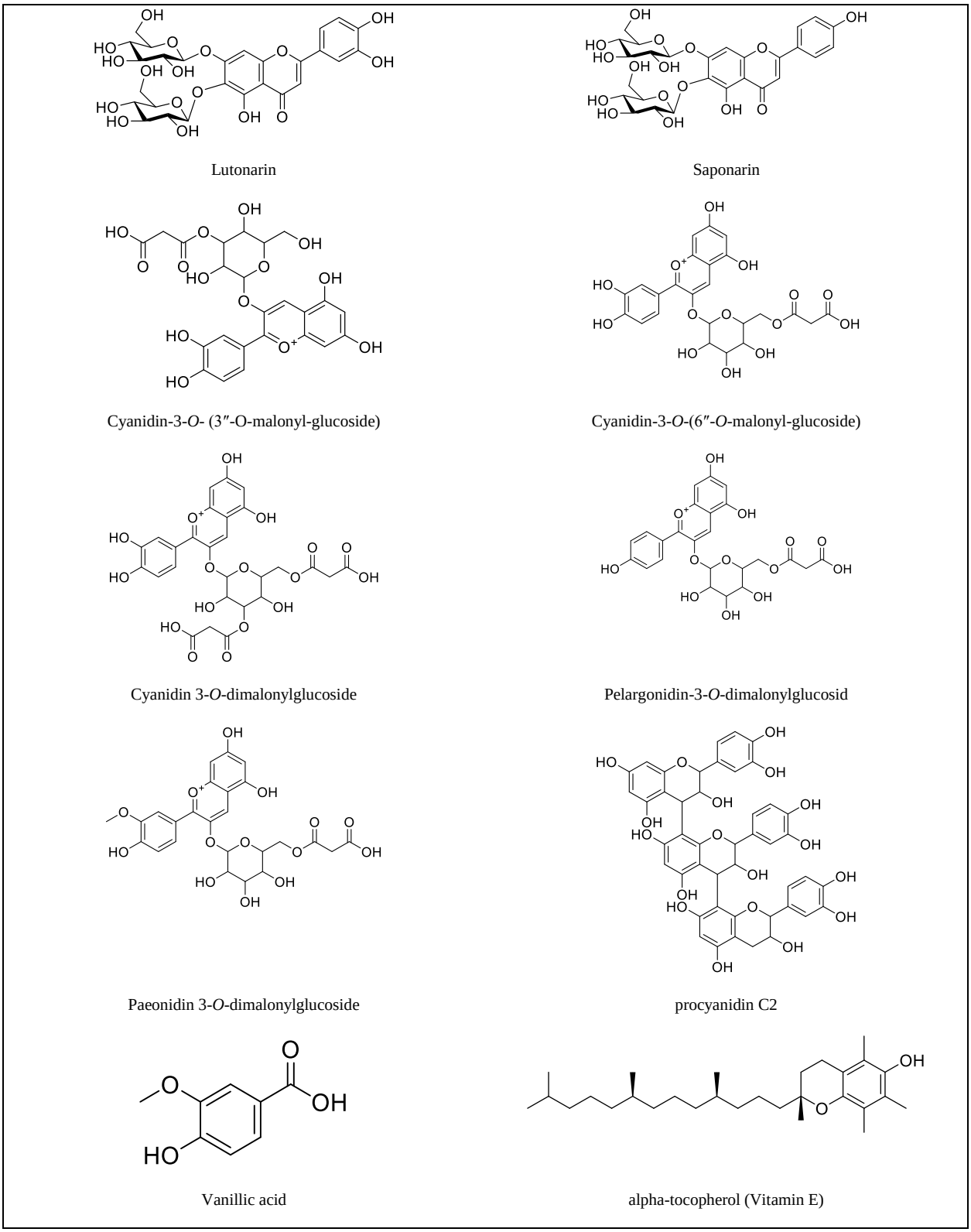

185 Fig. (2): Cont. Structures of the major compounds identified in Methanol Extract

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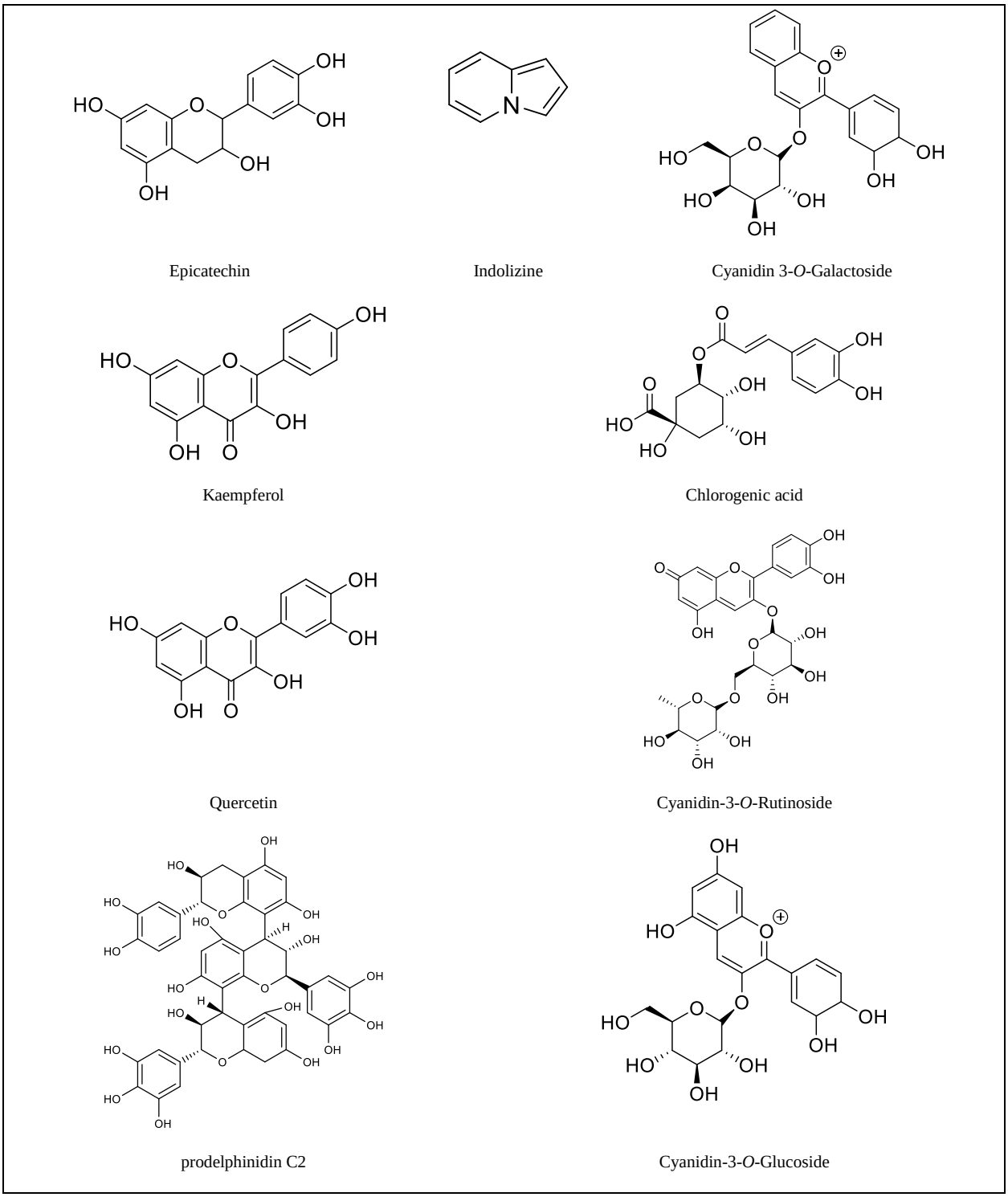

Fig. (2): Cont. Structures of the major compounds identified in Methanol Extract

4.3. Ethanol extract

Ethanol as a solvent is more or less similar to methanol, but it is less polar and less toxic. [48] The polarity of ethanol is usually increased by adding water to the pure ethanol. This results in the extraction of more bioactive phenolic compounds. Most studies used 70-80% ethanol/water for extraction of barley plant resulting in the identification of several phenolic compounds such as 1-*O*-sinapoyl-beta-d-glucose, 4-Hydroxybenzoic acid, tricin, apigenin, Apigenin 7-alpha-l-arabinopyranosyl-(1->6)-glucoside, Lutonarin, Rutin, Saponarin, Sinapic acid, rosmarinic acid, luteolin and vanillic acid. [49, 50] The antioxidant activity was tested using different assays such as (FRAP, ABTs, DPPH, and Intracellular Reactive Oxygen species), moreover, the anti-inflammatory activity was also carried out by measuring tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). Different reports showed that the major activities were found in ethanol extracts from different varieties. [39, 51, 52]

Few studies measured the activities of the isolated compounds from barley against the anti-inflammatory activity; Saponarin was tested against alcoholic fatty liver in rats, and it showed suppression of TNF-α secretion and maintenance of hepatic GSH. [53] β-glucan was prepared from the ethanol extract of highland barley obtained from China and assessed in an ethanol-induced gastric ulcer model in rats by determining stomach cytokines PGE2 and NO. The results showed that the pre-treatment with β-glucan could alleviate the gastric mucosal damage induced by ethanol. [54] Anti-wrinkle and antimicrobial in addition to behavioral study are also specific activities measured for the ethanolic extracts. [49, 50, 55] Lee *et al.* (2018) studied the ethanolic extract after its fermentation by lactic acid bacteria for testing its anti-oxidant activity, it showed more antioxidant activity after fermentation. [51] Few papers measured total anthocyanins, total phenolic content, total flavonoids, and total condensed tannins for the ethanolic extract of barley. The

studies revealed that barley extracts with high phenolic content showed the highest scavenging DPPH radicals. [52]

The activity as well as the separated compounds are summarized in table (1) and figure (3) shows the major structures identified from the ethanol extract.

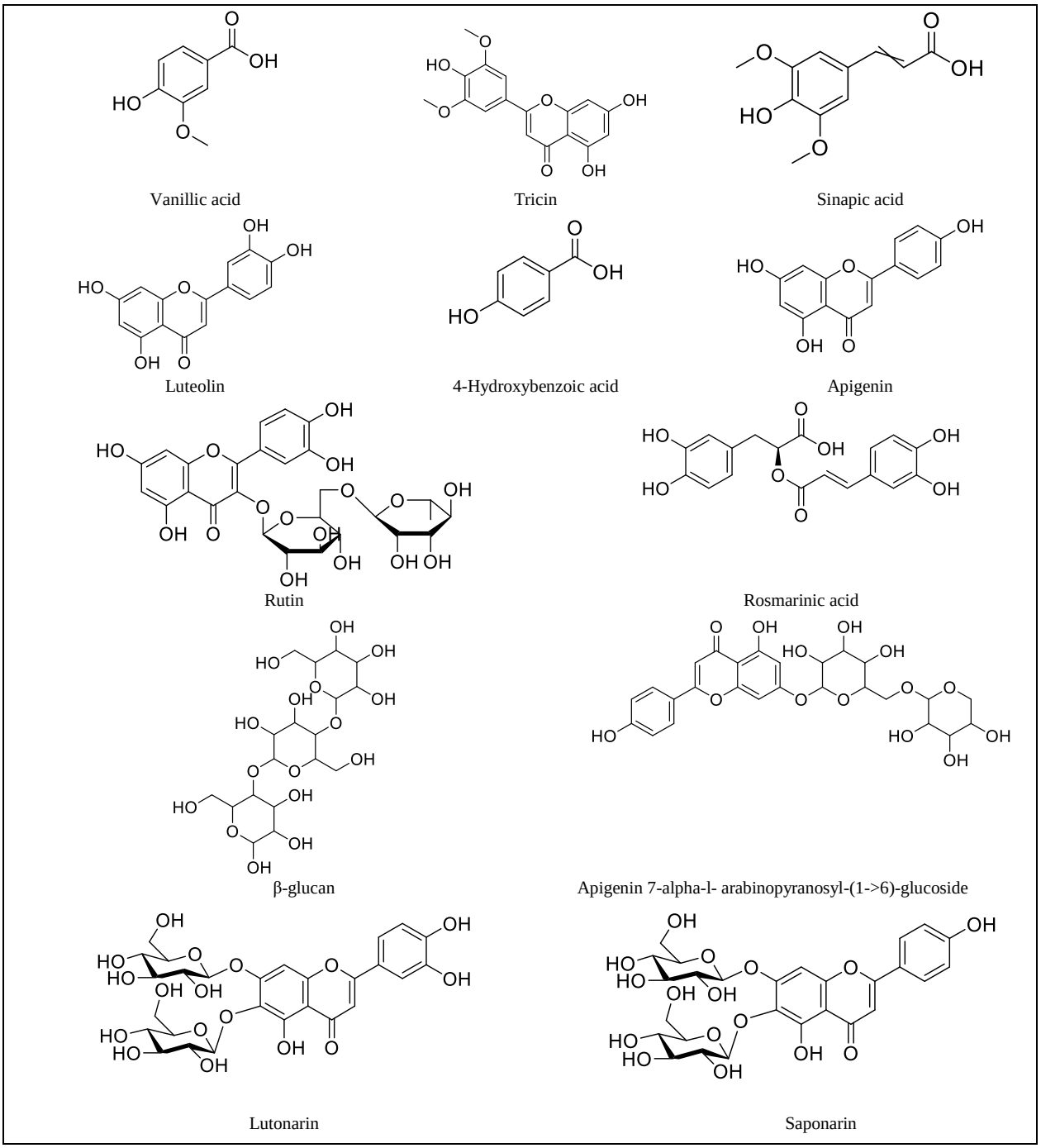

Fig. (3): Structures of the major compounds Identified in Ethanol Extract

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4.4. Acetone extract

Acetone as a solvent is a very useful extractant since it can dissolve many hydrophilic and lipophilic components. It is miscible with water, volatile, and has low toxicity. It is especially useful for the extraction of tannins and other phenolics. Numerous polyphenols were identified from the acetone extract of barley using LCMS and RP-HPLC like catechin-5-O-glucoside, prodelpinidin B3, catechuic acid, chlorogenic acid, catechin, caffeic acid, p-coumaric acid, ferulic acid, and chlorogenic acid from different locations like Australian, Hindmarsh and China verities. [56, 57] ORAC and cellular anti-oxidant activity assays were the major assays used to test the acetone extracts. [56, 57] The result from the ORAC assay showed higher anti-oxidant activity compared to the cellular anti-oxidant activity of four different varieties of barley from China. [56] Lee, Han, *et al.* (2013) extracted anthocyanins from the acetone extract of barley and showed a substantial ACE inhibitory and antioxidant effect. [58]

Table (1) summarizes the activity as well as the isolated chemicals and figure (4) shows the major structures of the identified compounds from the acetone extract.

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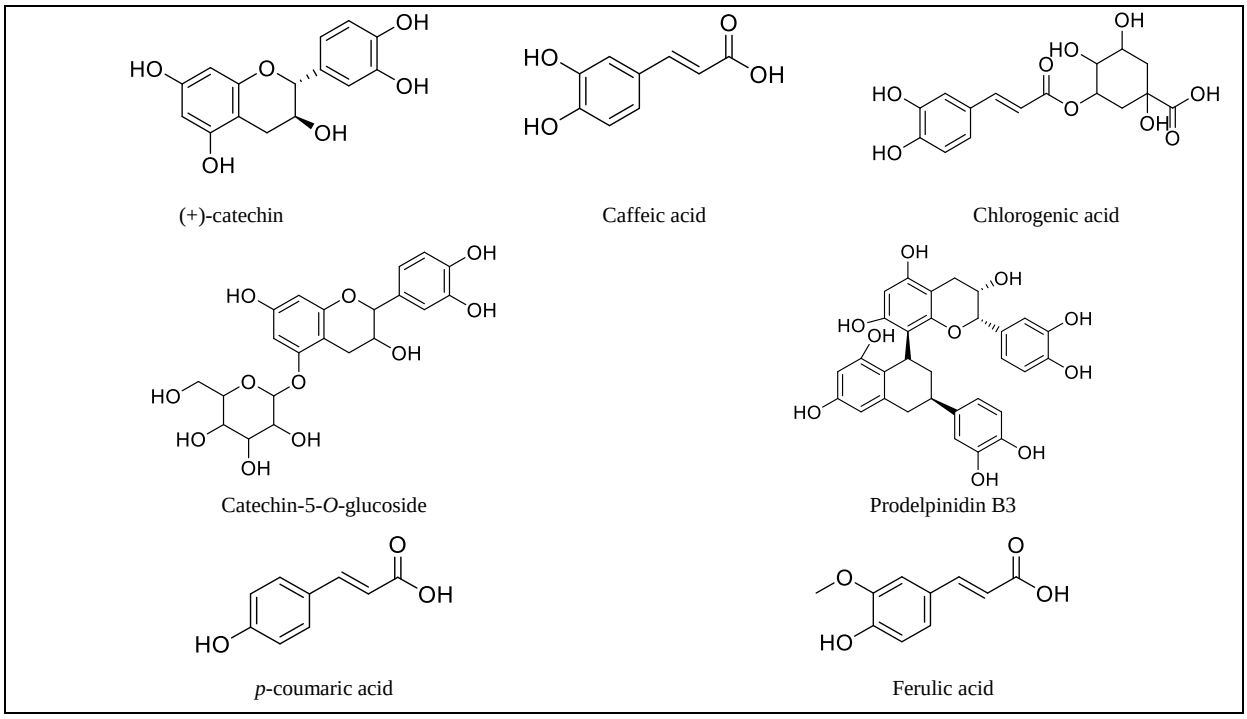

Fig. (4): Structures of the major compounds Identified in Acetone Extract

5. Conclusion

Cereal research has become a prominent topic in recent years, with barley attracting more attention in both the food and medical fields. β-Glucans, a primary fiber in barley, have been shown to lower plasma cholesterol, improve lipid metabolism, and lower the glycemic index. This review primarily focused on the type of solvents used in extraction, chemical characterization, and related biological activities of barley in this context. As per the previous reports, water, methanol, ethanol & acetone were used as solvents for barley extraction. Phenolic compounds and flavonoids were the major identified compounds from all used solvents. Antioxidant assays, total phenolic, and flavonoid content, and anti-inflammatory assays were the main biological activities studied from different solvents, while the anti-depressant activity assays were measured only from the water extract due to the safety of the water in *in-vivo* assays. A few studies compared the antioxidant activity of different solvent extracts, where they reported that the methanol extract had the highest activity compared to other solvents.

In conclusion, there aren't many studies comparing different biological activities among various solvent extracts. Therefore, we suggest that further studies should be done to provide evidence for the preference of a certain solvent over the other

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Keywords

Barley. β-glucan. Bioactive metabolites. Cereal crops. *Hordeum vulgare.*

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No support for the work was received.

Author Contributions

Omneya Eid: Investigation, Resources, Data Curation, Writing - Original Draft**, Wafaa Elkady:** Resources, Writing - Original Draft, Writing - Review & Editing**, Shahira ezzat:** Writing - Review & Editing, Visualization, and Supervision **Abeer El Sayed:** Writing - Review & Editing, Visualization, and Supervision **Essam Abd elsattar:** Writing - Review & Editing, Visualization, and Supervision

Author Declarations

The authors declare no conflict of interest for this study.

Author biographies

Omneya Eid received her bachelor's degree in pharmaceutical sciences from Future University in Egypt in 2013 and her master's degree from the faculty of pharmacy, at Cairo University in 2018. She is now studying for her Ph.D. at Cairo University. She is currently studying under the supervision of Prof. Essam Abdel-Sattar faculty of pharmacy, at Cairo University. Her research focus is to isolate and identify plant constituents using different chromatographic and spectral methods.

Dr. Wafaa Mostafa Elkady is an Associate Professor of Pharmacognosy. She graduated from the Faculty of Pharmacy at Helwan University in 2002. She earned a master's degree in Pharmacognosy in 2009. She holds PhDs in phytochemistry and Pharmacognosy 2015, both from the Faculty of Pharmacy at Helwan University. She is currently employed by the Faculty of Pharmacy Future University in Egypt as an Associate Professor and the department's head of Pharmacognosy and Medicinal Plants. Her professional interests focus on natural products in general. She published many research articles in the field of medicinal plants in different international journals.

Shahira M. Ezzat is one of top 2% scientists worldwide identified by Stanford University 2021 and 2022. In 2007, she received her Ph.D. in Pharmacognosy from Cairo University's Faculty of Pharmacy. She is positioned as a Professor and Head of Pharmacognosy Department, Faculty of Pharmacy, MSA University since September 2017, and as a Vice Dean for Community Services and Environmental Development since October 2021. She published about 140 articles and book chapters in the chemistry and biological activity of natural products. Her

main research field is the isolation of bioactive plant constituents and the elucidation of their structures using different methods.

Abeer Mohamed Ali El Sayed, Professor of natural product chemistry, Pharmacognosy department, faculty of pharmacy, Cairo University of Egypt. She earned a master's degree in Pharmacognosy in 1999. She holds PhDs in phytochemistry and Pharmacognosy 2010 from the faculty of pharmacy, Cairo university. She has skills and expertise in flavonoids, triterpenes, natural product chemistry, Phytochemicals, Herbal Medicine, Natural Product Drug Discovery, Compound Isolation, Phytochemical Analysis, Phytochemical Purification, Structure Elucidation, Natural Product Isolation. About 30 publications with H-index 11.

Prof Abdel-Sattar was born in 1957 and graduated from Faculty of Pharmacy, Cairo University in 1979. He finished his PhD in 1990 (Munster, Germany). In 2000, he got a JSPS post-doctor fellowship, Toyama University, Japan. He served as director of Pharmacognosy Department (2012-2017) and as director of Natural Product research center since 2016, Cairo University, as vice director of Higher Scientific Committee of Egyptian universities for promotion to professors (2013-2016), and a member of Toyama-Asia-Africa Pharmaceutical Network since 2016. He published more than 180 papers in the field of natural products. He granted and participated in more than 25 projects.

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Table (1): Summary of the identified compounds in addition to the biological activity of different barley cultivars:

Solvent used	Origin	Biological activity	Main compounds detected	Tool of chemical analysis	Ref.
80% methanol	Tunisia	antioxidant assays ABTs, DPPH, and FRAP			[59]
80% methanol	Pakistan.	Glutathione peroxidase activity determination, Superoxide dismutase activity determination and Statistical analysis			[11]
80% methanol	Korea	Anti-inflammatory			[40]
70% methanol	Korea	Anti-inflammatory in vivo- in vitro Measurement of NO and pro-inflammatory cytokines in cultures, NF-kB DNA-binding activity, The serum levels of TNF-a, IL-1b and IL-6	Benzeneacetic acid, Benzene-propanoic acid, Benzeneacetic acid, 4-hydroxy-3-methoxy Benzene-propanoic acid, 4-hydroxy-3-methoxy, 1-Propanone, 3-hydroxy-1-(4-hydroxy-3-) methoxyphenyl 7-Methoxy-4a-methyl-9,10-dihydro-2(4aH)-phenanthrenone	GC-MS analysis	[12]

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			2-Methoxy-4-vinyl phenol Phenol, 2,4-bis(1,1-dimethyl ethyl) 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl) Tetradecanoic acid Pentadecanoic acid, 14-methyl-, methyl ester n-Hexadecanoic acid, Hexadecanoic acid, methyl ester Octadecadienoic acid methyl ester, Octadecadienoic acid		
20% methanol		Anti microbial, Well methods, disc diffusion methods, and OD of broth culture			[47]
80% methanol	European and Syrian cultivars		75 compounds, mainly: apigenin, Luteolin, chrysoeriol	LCMS	[37]
Methanol	Copenhagen		Azelaic acid	UHPLC/MS/MS	[42]
80% methanol	Pakistan	antiglycation antioxidant activities (DPPH assay)	4-methoxy-5, 7-dihydroxy isoflavone(Biochanin A)	LCMS and GCMS	[38]

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			alpha-tocopherol (Vitamin E)		
70% methanol	Serbia	in vitro antioxidant capacity (DPPH- ABTs), antihyperglycaemic and anti-inflammatory activities	Gallic acid, p-hydroxybenzoic acid Protocatechuic acid, Vanillic acid Catechin, Epicatechin, Chlorogenic acid, Ferulic acid, Sinapic acid Myricetin, Quercetin, Kaempferol	HPLC	[33]
methanol	Nepal	(TPC), total flavonoid, DPPH determination of HRBC membrane stabilization method, Brine Shrimp Lethality Assay (BSLA) Comparing methanol to ethyl acetate and hexane extracts, it was found that methanol was much more effective at extracting polyphenolic chemicals.	Indolizine Phytol	GCMS	[45]
hexane					
ethyl acetate					
80 % Methanol	Spain		49 compounds procyanidin B3,prodelphinidin B3 catechin, catechin diglucoside, procyanidin C2, prodelphinidin C2	HPLC UPLC/MS/MS	[34]

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80% Methanol	Qinghai	Antioxidant capacity (DPPH-ABTS Hydroxyl radical scavenging activity-Superoxide anion scavenging activity)	156 compounds	LCMS	[44]
70% methanol 70% Acetone 70% ethanol + different methods for extraction	Morocco	(TPC), Total flavonoids (TFC), proanthocyanidins (PA) 70% methanol extract > 70% acetone extract ≈70% ethanol extract. Acetone extract had the best DPPH-scavenging activity. Compared to soxhlet and maceration, ultrasonic extraction was more effective			[46]

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80% methanol	Qinghai	Identify anthocyanins, DPPH, ABTs, Hydroxyl radical scavenging activity, Superoxide anion scavenging activity, Ferric reducing antioxidant power assay	Cyanidin-3-*O*-Glucoside, Cyanidin-3-*O*-Rutinoside, Cyanidin-3-Galactoside and other anthocyanins	ESI-MS	[35]
Methanol extract		LPS-induced upregulation of pro-inflammatory cytokines interleukin (IL)-6, Tumor necrosis factor (TNF)-α, the inflammatory enzyme cyclooxygenase-2 (COX-2), Inducible nitric oxide synthase (iNOS).	Lutonarin and Saponarin	UPLC-PDA	[41]
methanol	Tibet	Antioxidant activity (DPPH)	six compounds Cyanidin-3-glucoside Cyanidin-3-*O*-(3″-*O*-malonyl-glucoside), Cyanidin-3-*O*-(6″-*O*-malonyl-glucoside), Cyanidin 3-*O*-dimalonylglucoside, Pelargonidin-3-	UPLC/Q-TOF-MS	[36]

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			O-dimalonylglucoside, Paeonidin 3-*O*-dimalonylglucoside		
70% methanol	Algeria	Alkaloids, Flavonoids, Tannins, Saponins, and Coumarins Screening Total Phenolic Content, Total Flavonoid content, total Tanins Content, DPPH			[43]
80% methanol	Korea	anti-oxidant activity			[53]
acetone		DPPH radical scavenging capacity, superoxide radical scavenging capacity, and total antioxidant activity. The half maximal inhibitory concentration (IC50) of angiotensin I-converting enzyme (ACE	cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside cyanidin-3-(6″-succinyl) glucoside, cyanidin-3-(6″-succinyl) glucoside, peonidin-3-(6″-succinyl) glucoside, cyanidin derivative, peonidin derivative, cyanidin derivative peonidin derivative, cyanidin peonidin derivative, Meresse	LCMS	[58]

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80% chilled acetone		ORAC, Cytotoxicity (HepG2 human liver cancer cells) not good results	Chlorogenic acid, Catechin, Caffeic acid, *p*-Coumaric acid, Ferulic acid	RP-HPLC	[56]
30% ethanol	Korea	Anti-inflammatory(the suppression of TNF-α secretion and maintenance of hepatic GSH by barley sprouts extract, Cox 2, iNOs)	Saponarin	LC-MS/MS	[53]
95% Ethanol	China	decrease the level of interleukin-6 and tumor necrosis factor-alpha and increased level of prostaglandin E2, nitric oxide(*invivo*)	β-glucan		[54]
Ethanol	Korea	total polyphenol content, total flavonoid content, DPPH radical scavenging, superoxide dismutase-like activity, and tyrosinase inhibition			[51]

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70% ethanol	Korea	In vitro anti-wrinkle activity	Rosmarinic acid, Luteolin, Apigenin	UPLC–PDA–ESI–TOF–MS	[50]
80% ethanol	Italy	Total Anthocyanins, FRAP, ABTs			[52]
30% ethanol	Egypt	DPPH, Behavioral study			[55]
Water	Korea	Antioxidants (ABTs, DPPH, FRAP), TPC	p-coumaric acid, ferulic acid	HPLC	[28]
Water	USA	Anti-Diabetic, Total protein assay Anti-oxidant (DPPH and ABTS)	Gallic acid, protocatechuic acid, catechin, caffeic acid	HPLC	[19]
Water	USA	TPC, α-Glucosidase Inhibitory Assay, Maltase Inhibitory Activity Sucrase Inhibitory Activity, DPPH			[26]
Water	Tunisia	Antioxidant	*p*-coumaric acid, syringic acid, and other 17 compounds	LC-MS	[5]
Deionized Water	Brno	ABTs, DPPH			[6]

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water	Japan	Anti-depressant Restraint stress			[23]
Water	Poland	DPPH, Colon carcinoma, cancer cell proliferation inhibition			[27]
water	Poland	Polyphenolic Acids Determination, ABTS, DPPH, Cholinesterases Inhibition	Catechin, Epicatechin, Quercetin, Rutin, Kaempferitrin	HPLC	[20]
Water	Qingke	In vitro hypolipidemic activities antioxidant activities			[22]
Water	Egypt	Renal cell culture and cytotoxicity assay, DPPH, ABTS, inflammatory mediators, and kidney injury molecule-1 (KIM-1)	Vanillic acid, Syringic acid, *p*-Coumaric acid, Ferulic acid, Ellagic acid	HPLC	[21]
Water	USA	Ameliorate cellular oxidative stress (DPPH), total phenolic content, H_2O_2-induced oxidative stress assay, Cytotoxicity analysis using live-cell and propidium iodide exclusion assay, Analysis of Akt,			[29]

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		MAPK/ERK and STAT5 phosphorylation via Western blot			
Water	Korea	(lactose dehydrogenase (LDH), cytotoxicity detection assay	Polysaccharides		[22]
Water	Iran	FRAP			[25]
Water	India	FRAP, ferrous ion-chelating potential, DPPH, ABTS	β-glucan		[18]
water	China	preventing chronic inflammation in cardiovascular diseases, Anti-oxidant (ORAC-FRAP)			[30]
water	China	DPPH and NO radical scavenging activity assays			[17]