Review

The Ethnopharmacological, Phytochemical and Pharmacological Review of *Euryale ferox*, a Medicine Food Homology Species

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Abstract: *Euryale ferox*, which belongs to the family of Nymphaeaceae, has been widely distributed in China, India, Korea, and Japan. The seeds of *E. ferox* (EFS) have been categorized as superior food for 2000 years in China, based on its abundant nutrients including polysaccharides, polyphenols, sesquineolignans, tocopherols, cyclic dipeptides, glucosylsterols, cerebrosides, and triterpenoids. These constituents exert multiple pharmacological effects, such as antioxidant, hypoglycemic, cardioprotective, antibacterial, anticancer, antidepression, and hepatoprotective properties. There are very few summarized reports on *E. ferox*, albeit with its high nutritional value and beneficial activities. Therefore, we collected the reported literatures (since 1980), medical classics, databases, and pharmacopeia of *E. ferox*, and summarized the botanical classification, traditional uses, phytochemicals, and pharmacological effects of *E. ferox*, which will provide new insights for the further research and development of EFS-derived functional products.

Keywords: *Euryale ferox*; traditional medicine; phytochemical constituents; pharmacological effects

1. Introduction

Euryale ferox seed (EFS) is a typical representative of "a medicine food homology species", as described in the Huangdi Nei Jing Tai Su (黄帝内经太素): "Eating it as food on an empty stomach, and taking it as medicine for patients" [1,2]. EFS is the dried seeds of the *E. ferox* plant (**Figure 1A**), and is widely distributed in Bangladesh, Myanmar, New Zealand, Russia, Thailand, and parts of East Asia [3,4]. In China, EFS was first described in "Shen Nong's Classic of the Materia Medica" (Shén Nóng Běn Cǎo Jīng, 神农本草经) [5]. EFS, also known as Foxnut, Lotus seeds, Gorgon nuts, and Phool Makhana, is spherical, commonly broken grains, 5-8 mm in diameter when intact, with brownish red inner seed coat on the surface, yellowish white at the other end (**Figure 1B**) [6–8].

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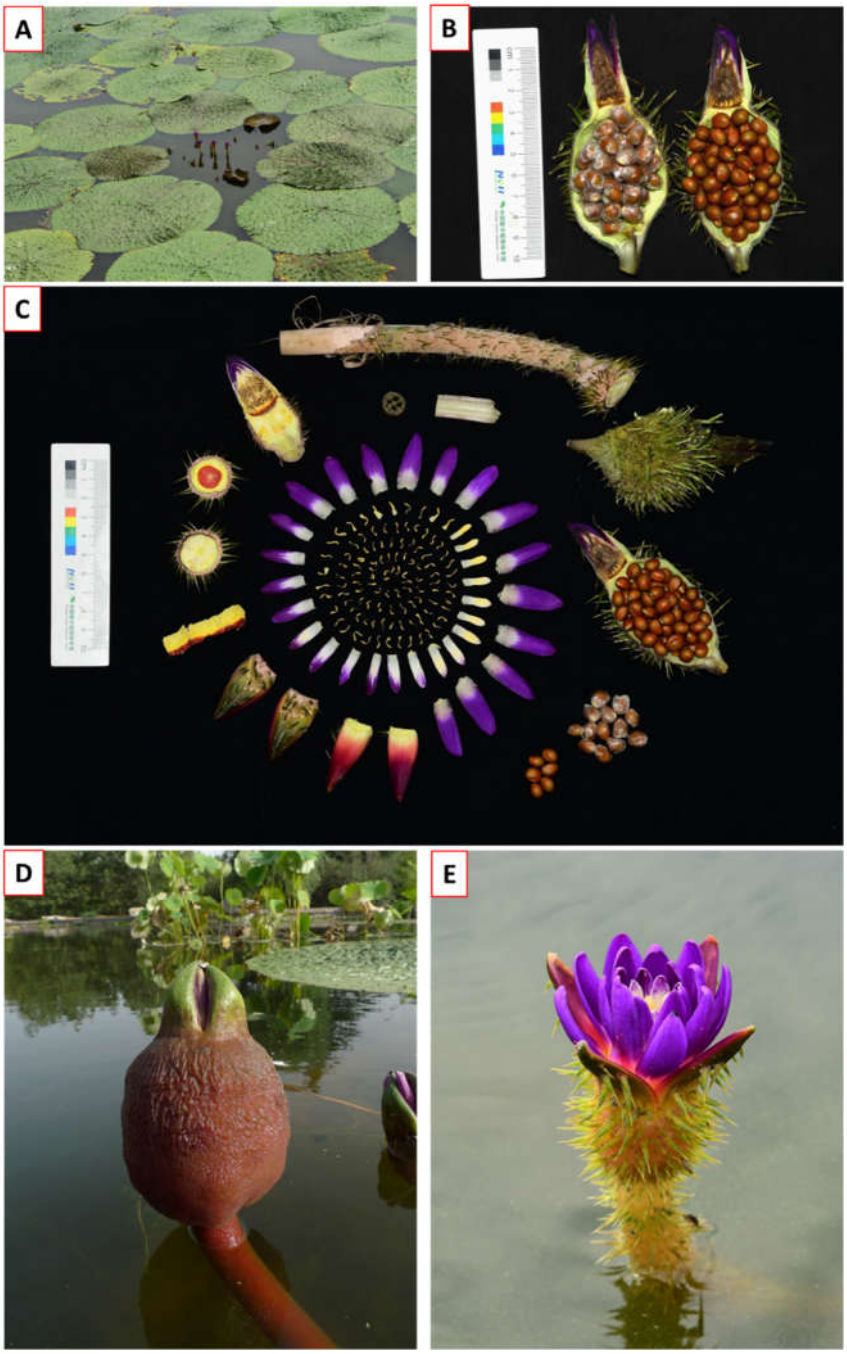

Figure 1. The biotope and morphological characteristic of *E. ferox*.

As a folk medicine in China for thousands of years, EFS is mainly used to reinforce the kidney, invigorate essence, and tonify the spleen to arrest diarrhea. It is commonly used to treat spermatorrhea, gonorrhea, dysmenorrhea, incontinence of urine, and diarrhea of the bowels [9]. In March 2002, the National Health Care Commission of the People's Republic of China embodied EFS as one of the new herbal medicines on the list of medicinal and food ingredients, with the stipulation that it can only be used for both medicinal and food purposes within a limited range and dosage. The dried ripe seed is included in the Chinese Pharmacopoeia (2020 Edition) as a commonly used Chinese herbal medicine. According to the theory of Chinese medicine, it tastes sweet, bitter, and astringent, attributes to the spleen and kidney meridians [9]. EFS contains an assortment of chemical constituents including triterpenoids, sterols, flavonoids, phenylpropanoids, organic acids, essential oils, and polysaccharides, while triterpenoids and flavonoids are considered as major active components [8,10,11]. Modern pharmacological studies indicate that it exerts a wide range of bioactive activities, such as anti-tumour, anti-bacterial, anti-viral, anti-inflammatory, immunomodulatory,

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hypotensive, hypoglycaemic, hypolipidaemic, anti-oxidant, free radical scavenging, and hepatoprotective effects [13–23]. More importantly, its excellent qualities and remarkable efficacy have also been highlighted in clinical applications, which are mainly used for the treatment of cancer, hypertension, diabetes, pelvic inflammatory disease, thyroid disorders, and prostate disorders [24].

Although the constituents and bioactivities of EFS have been extensively investigated, the detail summary was not made. Thus, we here perform a comprehensive, in-depth, and systematic review of EFS's botany, traditional applications, phytochemistry, pharmacological effects, and toxicity in last 30 years, which will provide quotable evidences for future studies on EFS and contribute to shed further light on the biological activities and clinical applications of EFS.

2. Methodology

To retrieve information relevant to this review, an extensive literature search was conducted using Google Scholar, Web of science, PubMed, SciFinder, China National Knowledge Infrastructure (CNKI), and China Science and Technology Journal databases. The main keywords searched were "*E. ferox*", "Foxnut", "Lotus seeds", "Gorgon nuts", or "Phool Makhana". Subsequently, the keywords "chemical composition", "phytoconstituents/phytochemicals", "biological activity", "pharmacological activity", and "toxicology" were searched to refine the publications. *E. ferox*-related articles were retrieved from peer-reviewed journals worldwide range from 1980 to 2023. These articles were read thoroughly during the compilation and integration of the information in order to evaluate the authenticity and relevance of their information. The studies that lacking scientific names were excluded, as well as studies on the uses of EFS in fields other than the medical and nutritional sciences. All chemical structures were validated by Sci Finder and drawn using Chem Draw Ultra 15.0.

3. Botanical Studies of *E. ferox*

E. ferox is widely distributed to the tropical and subtropical regions of Asia and Southeast Asia. India, Japan, Korea, Bangladesh, and China are the main producing areas [6]. India contributes nearly 70-80% of the global EFS production. Generally, *E. ferox* is grown in stagnant water with a depth of 0.2-2.0 m, such as ponds and lakes (**Figure 1A**). It prefers warm and sunny weather, and intolerant to cold and drought. The suitable temperature is range from 20 to 30°C, while a fertile soil with sufficient organic matter is required [6,25]. *E. ferox* consists of 8-9 leaves, while the bright purple flowers are arranged in alternating rows and interlaced like an octopus. The roots are long, fleshy, fibrous, usually in 2-3 clusters with numerous stomata, while the seeds are round and tuberous with diameter 0.5-1.5 cm (**Figure 1C**) [7]. The whole plant of *E. ferox* is edible except for the leaves [6]. In China, *E. ferox* are divided into southern and northern varieties. Northern *E. ferox*, also known as prickly *E. ferox*, is a wild species with purple flowers. It is mainly distributed in Hongze lake and Baoying lake of Jiangsu province (**Figure 1E**). Southern *E. ferox*, also known as Su Qian, is a variety of northern *E. ferox* after artificial domestication and cultivation, with larger leaves and white, red, or purple flowers (**Figure 1D**). Nevertheless, the southern *E. ferox* is mainly used for food and export, while the northern *E. ferox* for medicinal application [1,4].

It is worth noting that the growing and processing of *E. ferox* is a laborious and demanding work. To maintain plant-to-plant spacing, thinning seedlings is required after planting, while seeds collecting from the pond is needed during harvesting. Subsequently, processing including storage, cleaning, grading, heating, and tempering are required, all of which are performed manually. The whole operation is therefore a strenuous and painful work from planting to harvest.

4. Traditional Medicinal Uses of *E. ferox*

Due to the unique natural conditions and long-term practice in different regions, it has led to versatile lifestyle and unique experiences in the treatment of certain diseases. EFS are generally used as an economic crop in India, Japan, Korea, Bangladesh, and other Southeast Asian countries [26]. In China, EFS was recorded for the first time in "Shen Nong's Classic of the Materia Medica" and was described as "sweet and astringent tastes, and used for dampness and paralysis, pain in the lumbar spine and knees, tonifying and removing malignant diseases, benefiting the essence, strengthening the will, and making the ears and eyes wise" [5], and most of the subsequent records followed this statement with modifications. Current Chinese Pharmacopoeia (2020 Edition) recorded EFS as "sweet, astringent, and flat, attributes to the spleen and kidney meridians, benefiting the kidney and consolidating sperm, tonifying the spleen

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and inhibiting diarrhoea, eliminating dampness and arresting leucorrhea. It is used for spermatorrhea, enuresis and frequent urination, splenoasthenic diarrhea, and leucorrhea" [9]. In India, EFS is known as makhana, and is applied in Ayurvedic medicines for treating diseases including bile disorders, persistent diarrhea, kidney disorders, rheumatic disorders, excessive leucorrhea, hepatic dysfunctioning, etc [27]. While in Japan, EFS is recorded in Kampo medicine for improving metabolic arthritis, urinary incontinence, and leucorrhea. In addition to EFS, the whole plant of *E. ferox* can be used as food or medicine. As early as the report in the Compendium of Materia Medica in Ming dynasty, China, the stems, leaves, and roots of *E. ferox* were applied to treat different diseases. To ascertain the efficacy and traditional uses of *E. ferox*, we summarized the records in ancient herbal works or research reports (**Table 1**).

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Table 1 The traditional uses of *E. ferox*

Parts	Herbal records	Dynasty/Country	Effects	Ref
Seeds	Shen Nong's Classic of the Materia Medica (Shén Nóng Běn Cǎo Jīng, 神农本草经)	Eastern Han Dynasty, AD 25-220, China	Eliminating dampness, easing backache and knees pain, tonifying and removing malignant diseases, benefiting the essence, strengthening the will, and making the ears and eyes wise.	[5]
	The Compendium of Materia Medica (Běn Cǎo Gāng Mù, 本草纲目)	Ming Dynasty, AD 1578, China	Quenching thirst and benefiting the kidney, treating urinary incontinence, spermatorrhea, and leucorrhea.	[28]
	The Song of Medicinal Properties and four hundred flavours (Yào Xìng Gē Kuò Sì Bǎi Wèi Bái Huà Jiě, 药性歌括四百味)	Ming Dynasty, AD 1581, China	Benefitting the essence, relieving soreness of the waist and knees, arresting seminal emission.	[29]
	Leigong Concocted Medicinal Annotation (Léi Gōng Páo Zhì Yào Xìng Jiě,雷公炮制药性解)	Ming Dynasty, AD 1622, China	Tonifying the spleen and stomach, benefitting the essence, improving visual and auditory acuity, and amnesia.	[29]
	Essentials of Chinese Materia Medica (Běn Cǎo Bèi Yào, 本草备要)	Kangxi XXXIII, AD 1694, China	Strengthening the kidney and benefiting the essence, tonifying the spleen and eliminating dampness. Treating diarrhea with turbidity and spermatorrhea.	[29]
	Chinese Pharmacopoeia	AD 2020, China	Benefiting the kidney and consolidating sperm, tonifying the spleen and inhibiting diarrhoea, eliminating dampness and arresting leucorrhea, improving spermatorrhea, enuresis and frequent urination, splenoasthenic diarrhea, and leucorrhea.	[9]
	Traditional Medical & Pharmaceutical Database	Japan	Improving metabolic arthritis, urinary incontinence, and leucorrhea, easing waist pain.	-
	Ayurveda and Unani system	India	Improving rheumatic and bile disorders, against dysmenorrhea and exerting spermatogenic properties.	[27]

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Stems	The Compendium of Materia Medica (Běn Cǎo Gāng Mù, 本草纲目)	Ming Dynasty, AD 1578, China	Quenching irritability and thirst, eliminating asthenia-heat syndrome.	[28]
Leaves	The Compendium of Materia Medica (Běn Cǎo Gāng Mù, 本草纲目)	Ming Dynasty, AD 1578, China	Treating retained placenta and haematemesis.	[28]
Roots	The Compendium of Materia Medica (Běn Cǎo Gāng Mù, 本草纲目)	Ming Dynasty, AD 1578, China	Improving swollen testicles, abdominal pain due to stagnation of vital energy.	[28]

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5. Phytochemistry

Various phytochemicals are isolated and determined in *E. ferox*, which can be classified into polysaccharides, polyphenols, cyclic dipeptides, cerebrosides, cholesterols, tocopherols, and triterpenoids based on the chemical properties. The information of secondary metabolites in *E. ferox* has been investigated, and 589 secondary chemicals were obtained from leaves, petiole, seed shell, fruit peel, and seed kernels of *E. ferox* by UHPLC-MS/MS analysis, with polyphenols occupying 87%. Glycosylated flavonoids are accumulated significantly in the leaves, polyphenols are abundant in seed shell, phenolic acids are attributed in fruit peel. While, flavonoids are variable among the five tissues [30]. All of those reported phytoconstituents are summarized in **Table 2** and their structures are presented in **Figures 2-12**.

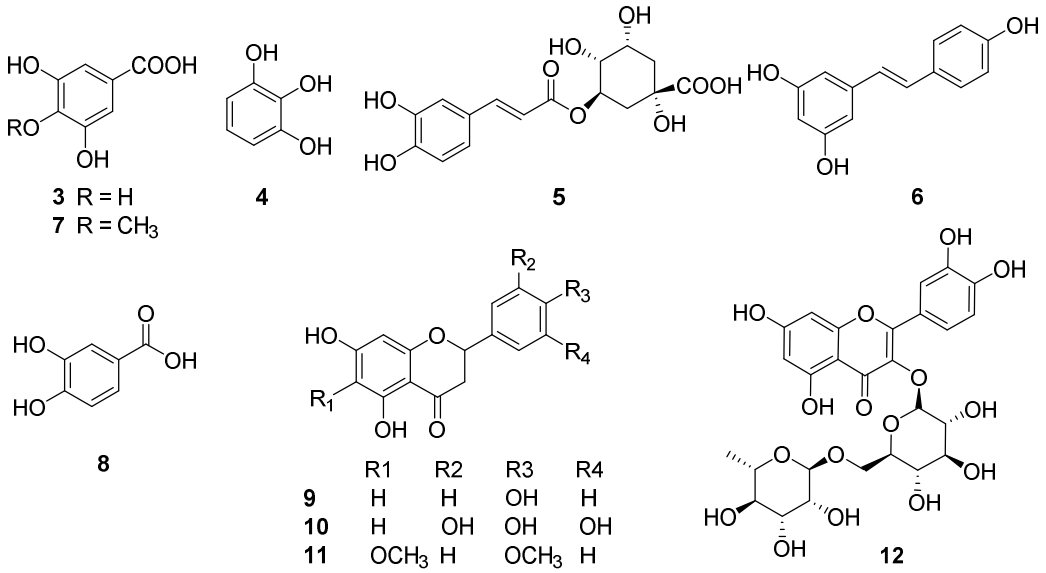

Figure 2. Chemical structures of polyphenols and flavonoids of E. ferox.

Table 2. Phytochemical compounds identified from the seeds of E. ferox.

No.	Compounds	Molecular formula	Type	Plant part	Ref
1	EPJ	-	Polysaccharide	Seeds	[31]
2	EFSP-1	-	Polysaccharide	Seeds	[32]
3	Gallic acid	$C_7H_6O_5$	Polyphenol	Seed shells, Seeds	[12,16,36]
4	Pyrogallol	$C_6H_6O_3$	Polyphenol	Seed shells	[36]
5	Chlorogenic acid	$C_{16}H_{18}O_9$	Polyphenol	Seed shells	[36]
6	Resveratrol	$C_{14}H_{12}O_3$	Polyphenol	Seeds	[16]
7	4-*O*-methyl gallic acid	$C_8H_8O_5$	Polyphenol	Seeds	[37]
8	Protocatechuic acid	$C_7H_6O_4$	Polyphenol	Seeds	[12]
9	Naringenin	$C_{15}H_{12}O_5$	Flavonoid	Seed shells, Seeds	[18,38,39]
10	Dihydrotricetin	$C_{15}H_{12}O_7$	Flavonoid	Seed shells	[18]
11	Pectolinarigenin	$C_{17}H_{16}O_6$	Flavonoid	Seeds	[12]
12	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid	Seed shells	[36]
13	Cyclo(Pro-Ser)	$C_8H_{12}O_3N_2$	Cyclodipeptide	Seeds	[38]
14	Cyclo(Ile-Ala)	$C_9H_{16}O_2N_2$	Cyclodipeptide	Seeds	[38]

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15	Cyclo(Leu-Ala)	$C_9H_{16}O_2N_2$	Cyclodipeptide	Seeds	[38]
16	Cyclo(Phe-Ser)	$C_{12}H_{14}O_3N_2$	Cyclodipeptide	Seeds	[40]
17	Cyclo(Ala-Pro)	$C_8H_{12}O_2N_2$	Cyclodipeptide	Seeds	[40]
18	Cyclo(Phe-Ala)	$C_{12}H_{14}O_2N_2$	Cyclodipeptide	Seeds	[40]
19	N-*α*-hydroxy-*cis*-octadecaenoyl-l-*O*-*β*-glucopyranosylsphingosine	$C_{42}H_{79}O_9N$	Cerebroside	Rhizomes with adventitious root	[41]
20	Peracetylated cerebroside	$C_{54}H_{91}O_{15}N$	Cerebroside	Rhizomes with adventitious root	[41]
21	(2*S*,3*R*,4*E*,8*E*,2′*R*)-1-*O*-(*β*-glucopyranosyl)-N-(2′-hydroxydocosanoyl)-4,8-sphingadienine	$C_{44}H_{83}O_9N$	Cerebroside	Seeds	[42]
22	(2S,3R,4E,8E,2′R)-1-O-(β-glucopyranosyl)-N-(2′-hydroxytetracosanoyl)-4,8-sphingadienine	$C_{46}H_{87}O_9N$	Cerebroside	Seeds	[42]
23	*β*-sitosterol	$C_{29}H_{50}O$	Steroid	Seeds	[12]
24	Daucosterol	$C_{35}H_{60}O_6$	Steroid	Seeds	[12]
25	Fucosterol	$C_{29}H_{48}O$	Steroid	Seeds	[37]
26	24-methylcholest-5-enyl-3*β*-*O*-pyranoglucoside	$C_{43}H_{49}O_{10}$	Steroid	Rhizomes with adventitious root	[43]
27	24-ethylcholest-5-enyl-3*β*-*O*-pyranoglucoside	$C_{44}H_{51}O_{10}$	Steroid	Rhizomes with adventitious root	[43]
28	24-ethylcholesta-5,22*E*-dienyl-3*β*-*O*-pyranoglucoside	$C_{44}H_{49}O_{10}$	Steroid	Rhizomes with adventitious root	[43]
29	2β-hydroxybetulinic acid 3β-caprylate	$C_{38}H_{62}O_5$	Pentacyclic triterpene	Seeds	[44]
30	2β-hydroxybetulinic acid 3β-oleiate	$C_{48}H_{80}O_5$	Pentacyclic triterpene	Seeds	[45]
31	*α*-tocopherol	$C_{29}H_{50}O_2$	Tocopherol	Seeds	[38,46,47]
32	β-tocopherol	$C_{28}H_{48}O_2$	Tocopherol	Seeds	[38]
33	δ-tocopherol	$C_{27}H_{46}O_2$	Tocopherol	Seeds	[38]
34	Ferotocotrimer C/E	$C_{86}H_{142}O_6$	Tocopherol	Seeds	[42, 48]
35	Ferotocotrimer D	$C_{86}H_{142}O_6$	Tocopherol	Seeds	[42]
36	Tocopherol trimer IVa	$C_{87}H_{144}O_6$	Tocopherol	Seeds	[42]
37	Tocopherol trimer IVb	$C_{87}H_{144}O_6$	Tocopherol	Seeds	[42]
38	Ferotocodimer A	$C_{58}H_{98}O_5$	Tocopherol	Seeds	[48]
39	Euryalin A	$C_{31}H_{38}O_{10}$	Lignan	Seeds	[39]
40	Euryalin B	$C_{30}H_{36}O_9$	Lignan	Seeds	[39]

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41	Euryalin C	$C_{32}H_{40}O_{12}$	Lignan	Seeds	[39]
42	rel-(2R,3β)-7-*O*-methylcedrusin	$C_{20}H_{24}O_6$	Lignan	Seeds	[39]
43	syringylglycerol-8-*O*-4-(sinapyl alcohol) ether	$C_{23}H_{30}O_9$	Lignan	Seeds	[39]
44	(1*R*,2*R*,5*R*,6*S*)2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane	$C_{20}H_{22}O_6$	Lignan	Seeds	[39]
45	(+)-syringaresinol	$C_{22}H_{26}O_8$	Lignan	Seeds	[39]
46	Buddlenol E	$C_{31}H_{36}O_{11}$	Lignan	Seeds	[18, 39]
47	(+)-Isolariciresinol 9'-*O*-glucoside	$C_{26}H_{34}O_{11}$	Lignan	Seeds	[38]
48	3-(4-hydroxy-3-methoxybenzyl)-4-[(7'R),5'-dihydroxy-3'-methoxybenzyl] tetrahydrofuran	$C_{20}H_{24}O_6$	Lignan	Seeds	[37]
49	Furfural	$C_5H_4O_2$	Essential oil	Seeds	[46]
50	Pentanoic acid	$C_5H_{10}O_2$	Essential oil	Seeds	[46]
51	2-methyl-3-pentanone	$C_6H_{12}O$	Essential oil	Seeds	[46]
52	5-methyl-2-furancarboxaldehyde	$C_7H_{14}O$	Essential oil	Seeds	[46]
53	Hexanoic acid	$C_6H_{12}O_2$	Essential oil	Seeds	[46]
54	4, 4, 8-trimethyl-non-7-en-2-one	$C_{12}H_{22}O$	Essential oil	Seeds	[46]
55	1-(2-butoxyethoxy)- 2-propanol	$C_9H_{20}O_3$	Essential oil	Seeds	[46]
56	Phenol	C_6H_6O	Essential oil	Seeds	[46]
57	2-methylphenol	C_7H_8O	Essential oil	Seeds	[46]
58	4-methylphenol	$C_7H_8O_2$	Essential oil	Seeds	[46]
59	4-ethylphenol	$C_8H_{10}O$	Essential oil	Seeds	[46]
60	Isocreosol		Essential oil	Seeds	[46]
61	4-ethylguaiacol	$C_9H_{12}O_2$	Essential oil	Seeds	[46]
62	2, 6-dimethoxyphenol	$C_8H_{10}O_3$	Essential oil	Seeds	[46]
63	4-methoxy-2, 3, 6-trimethylphenol	$C_{10}H_{14}O_2$	Essential oil	Seeds	[46]
64	3, 4-dimethoxytoluene	$C_9H_{12}O_2$	Essential oil	Seeds	[46]
65	3-tert-butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	Essential oil	Seeds	[46]
66	1, 2, 3-trimethoxybenzene	$C_9H_{12}O_3$	Essential oil	Seeds	[46]

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67	[3.1.1] hept-3-en-2-one, 4, 6, 6-trimethyl-bicyclo	$C_{10}H_{14}O$	Essential oil	Seeds	[46]
68	Butylated hydroxytoluene	$C_{15}H_{24}O$	Essential oil	Seeds	[46]
69	1S, 4R, 7R, 11R-1, 3, 4, 7-tetramethyltricyclo [5.3.1.0(4, 11)] undec-2-en-8-one	$C_{15}H_{22}O$	Essential oil	Seeds	[46]
70	2, 6-bis (1, 1-dimethylethyl)-2, 5-cyclohexadiene-1, 4-dione	$C_{14}H_{20}O_2$	Essential oil	Seeds	[46]
71	2-methylnaphthalene	$C_{11}H_{10}$	Essential oil	Seeds	[46]
72	Pentamethyl benzene	$C_{11}H_{16}$	Essential oil	Seeds	[46]
73	1-ethylidene-1H-indene	$C_{11}H_{10}$	Essential oil	Seeds	[46]
74	Tridecane	$C_{13}H_{28}$	Essential oil	Seeds	[46]
75	Pentadecane	$C_{15}H_{32}$	Essential oil	Seeds	[46]
76	Hexadecane	$C_{16}H_{34}$	Essential oil	Seeds	[46]
77	Dodecane	$C_{12}H_{26}$	Essential oil	Seeds	[46]
78	Tetradecane	$C_{14}H_{30}$	Essential oil	Seeds	[46]
79	Heptadecane	$C_{17}H_{36}$	Essential oil	Seeds	[46]
80	Octadecane	$C_{18}H_{38}$	Essential oil	Seeds	[46]
81	Nonadecane	$C_{19}H_{40}$	Essential oil	Seeds	[46]
82	Palmitic acid	$C_{16}H_{32}O_2$	Essential oil	Seeds	[46]
83	Linoleic acid	$C_{18}H_{32}O_2$	Essential oil	Seeds	[46]
84	Ethyl gallate	C_9 $H_{10}O_5$	Ester	Seeds	[12]
85	4-hydroxybenzylethyl ether	$C_8H_{10}O_2$	Ether	Seeds	[39]
86	5,7-dihydroxychromone	$C_9H_6O_4$	Ketone	Seeds	[12]
87	ω-hydroxypropioguaiacone	$C_{10}H_{12}O_4$	Ketone	Seeds	[39]
88	Coniferyl aldehyde	$C_{10}H_{10}O_3$	Aldehyde	Seeds	[39]
89	Trans-p-hydroxycinnamaldehyde	$C_9H_8O_2$	Aldehyde	Seeds	[39]
90	p-hydroxybenzaldehyde	$C_7H_6O_2$	Aldehyde	Seeds	[39]
91	p-hydroxybenzyl alcohol	$C_7H_8O_2$	Alcohol	Seeds	[39]
92	p-hydroxyphenethyl alcohol	$C_8H_{10}O_2$	Alcohol	Seeds	[39]
93	2-methoxybenzene-1,3-diol	$C_7H_8O_3$	Phenyl alcohol	Seeds	[39]
94	4-ethoxyphenol	$C_8H_{10}O_2$	Phenol	Seeds	[39]
95	Resorcinol	$C_6H_6O_2$	Phenol	Seeds	[37]
96	Alliin	$C_6H_{11}NO_3S$	Sulfoxide	Seeds	[16]
97	Adenosine	$C_{10}H_{13}N_5O_4$	Nucleoside	Seeds	[49]
98	Guanosine	$C_{10}H_{13}N_5O_5$	Nucleoside	Seeds	[49]
99	Cytidine	$C_9H_{13}N_3O_5$	Nucleoside	Seeds	[49]
100	Uridine	$C_9H_{12}N_2O_6$	Nucleoside	Seeds	[49]

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101	Inosine	$C_{10}H_{12}N_4O_5$	Nucleoside	Seeds	[49]
102	Thymidine	$C_{10}H_{14}N_2O_5$	Nucleoside	Seeds	[49]
103	2′-deoxyadenosine	$C_{10}H_{13}N_5O_3$	Nucleoside	Seeds	[49]
104	2′-deoxyguanosine	$C_{10}H_{13}N_5O_4$	Nucleoside	Seeds	[49]
105	2′-deoxycytidine	$C_9H_{13}N_3O_4$	Nucleoside	Seeds	[49]
106	2′-deoxyuridine	$C_9H_{12}N_2O_5$	Nucleoside	Seeds	[49]
107	2′-deoxyinosine	$C_{10}H_{12}N_4O_4$	Nucleoside	Seeds	[49]
108	Xanthine	$C_5H_4N_4O_2$	Nucleobase	Seeds	[49]
109	Hypoxanthine	$C_5H_4N_4O$	Nucleobase	Seeds	[49]
110	Thymine	$C_5H_6N_2O_2$	Nucleobase	Seeds	[49]
111	Adenine	$C_5H_5N_5$	Nucleobase	Seeds	[49]
112	Cytosine	$C_4H_5N_3O$	Nucleobase	Seeds	[49]

5.1. Polysaccharides

Polysaccharides are one of the main components of E. ferox that exert multiple pharmacological activities [10]. A polysaccharide named EPJ (**1**) was isolated from EFS by DEAE-52 cellulose chromatography and Sephadex G-100 column, which is mainly composed of glucose and rhamnose with a molar ratio of 5.46:1, and the molecular weight was determined to 15.367 kDa [31]. Zhang et al. obtained a novel polysaccharide EFSP-1 (**2**) from EFS by DEAE sepharose FF and Superdex™ 75 gel chromatography, which was mainly composed of (1→4)-*α*-D-Glcp with branches substituted at O-6 and terminated with T-*α*-D-Glcp. The structure of EFSP-1 was characterized by NMR, FT-IR, and GC-MS [32]. The high starch content (72.27-83%) made E. ferox into a superfood, while resistant starch has gained widespread focus for its physiological functions. A type 3 resistant starch (RS3) was isolated from EFS, and it belongs to B + V type crystal and exerts high thermal stability [33]. Moreover, amylopullulanase treated E. ferox flour promoted the content of resistant starch, and against the release of glucose during in vitro digestibility analysis [34].

5.2. Polyphenols and Flavonoids

Polyphenol is a class of secondary metabolites with a polyphenolic structure widely present in E. ferox, mainly existed in the skin, roots, leaves, and fruits. An ultrasonic-assisted extraction technology was performed for the extraction of phenolic compounds from E. ferox seed shells, and three polyphenols and one flavonoid were determined by HPLC analysis (**3-5**, **12**). In addition, resveratrol (**6**) was detected in EFS extract, and an antioxidative compound 4-O-methyl gallic acid (**7**) with protocatechuic acid (**8**) were isolated from the ethyl acetate extract of EFS. Dihydroflavonoids are the most reported flavonoids in the seeds of E. ferox (**9-11**). The targeted flavonoid metabolome was determined to explore the dynamic changes of flavonoid biosynthesis by LC-ESI-MS/MS analysis, a total of 129 flavonoid metabolites were identified, including 11 flavanones, 8 dihydroflavanols, 16 flavanols, 29 flavonoids, 3 isoflavones, 12 anthocyanins, 29 flavonols, 6 flavonoid carbosides, 3 chalcones, and 13 proanthocyanidins [35]. However, these compounds were inferred by mass spectrometry and the reliability needs to be further verified. The structures of isolated polyphenols and flavonoids are shown in **Figure 2**.

5.3. Cyclic peptides

Cyclic peptides are a kind of cyclo-compounds composed of common and uncommon amino acids. Due to the specific properties such as good target selectivity, binding affinity, and low toxicity, cyclic peptides become attractive lead compounds for drug development [50]. Multiple bioactive activities have been reported including antimicrobial, anti-infection, anti-tumors, anti-chronic kidney diseases, anti-diabetes, and memory improvement [51, 52]. Using thin-layer in situ chemical reactions, several cyclic dipeptides (**13-18**) have been isolated from EFS. The isolated cyclic peptides are shown in **Table 2** and **Figure 3**.

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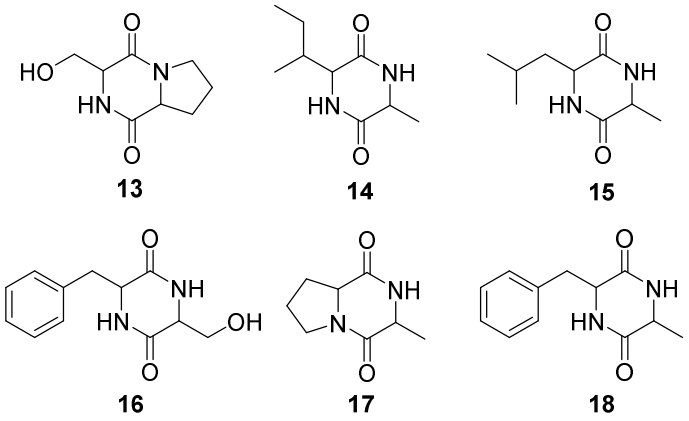

Figure 3. Chemical structures of cyclic peptides of E. ferox.

5.4. Cerebrosides

Cerebrosides are neutral chemicals that consist of a monosaccharide and ceramide bound by a β-glycosidic bond to the C1 of esfingol. As an important components of cell membrane in the nervous system, cerebrosides play important roles in regulating membrane dynamics and forming the internal structures. Four novel cerebrosides have been elucidated in the rhizome with adventitious root of *E. ferox* (**19**, **20**) and EFS (**21**, **22**), respectively [41, 42]. The structures of cerebrosides are shown in **Figure 4**.

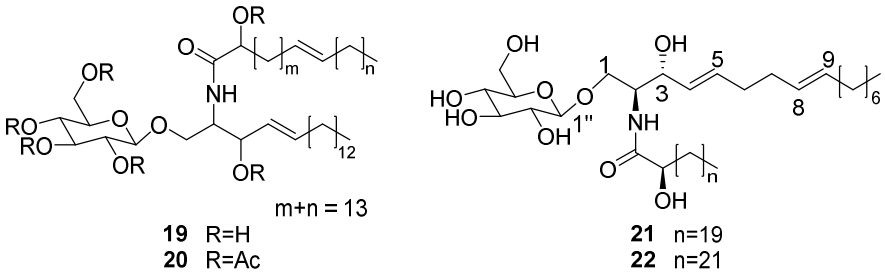

Figure 4. Chemical structures of cerebrosides of *E. ferox*.

5.5. Steroids and Pentacyclic Triterpenes

Steryl glycosides (SGs) and acylated steryl glycosides (ASGs) are two major derivatives of sterols. Steroidal glycolipids are considered to be unique glycolipids that play an important role in the structure of cells. Three sterols, *β*-sitosterol (**23**), daucosterol (**24**), and fucosterol (**25**) were isolated from EFS ethyl acetate extract [12, 37]. While, three glucosylsterols named 24-methylcholest-5-enyl-3*β*-*O*-pyranoglucoside (**26**), 24-ethylcholest-5-enyl-3*β*-*O*-pyranoglucoside (**27**), and 24-ethylcholesta-5,22*E*-dienyl-3*β*-*O*-pyranoglucoside (**28**) were obtained from the rhizomes with adventitious roots of *E. ferox* [43].

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Triterpenoids are of great interest to researchers owing to their wide range of biological activities. Gong investigated the contents of triterpenoids in 70% ethanol extract of *E. ferox* seed shell using vanillin-perchloric acid method, the total triterpenoids are up to 36.7% [53]. In order to investigate the putative active compounds responsible for antidiabetic, antioxidant, and antihyperlipidemic in EFS, Ahmed et al. obtained two novel triterpenoids, 2β-hydroxybetulinic acid 3β-oleiate (**29**) and 2β-hydroxybetulinic acid 3β-caprylate (**30**), from the ethyl acetate extract [44,45]. The specific information and structures of each compound are shown in **Table 2**, **Figure 5**.

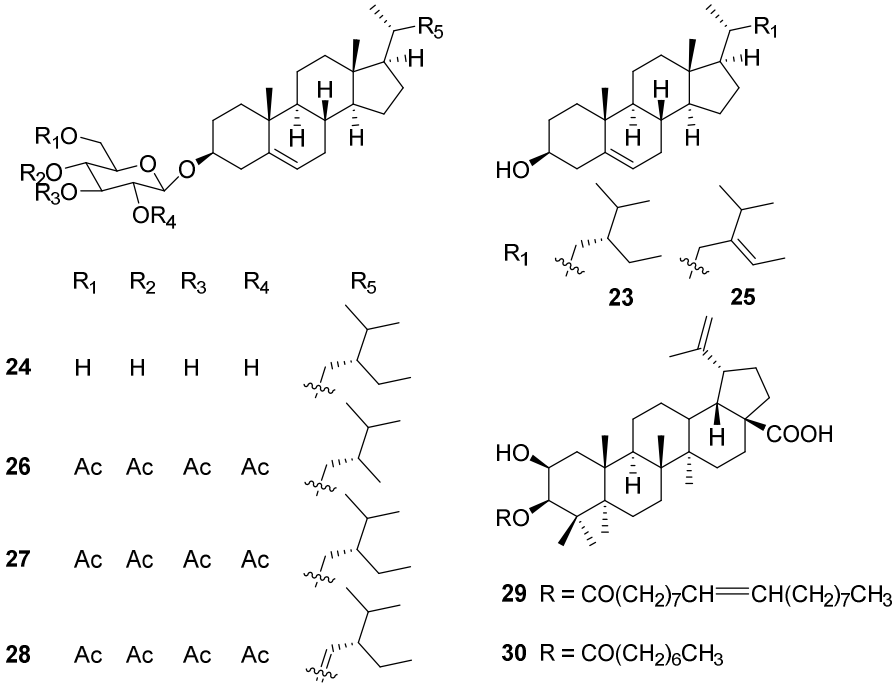

Figure 5. Chemical structures of steroids and pentacyclic triterpenes of *E. ferox*.

5.6. Tocopherol

Tocopherols are a series of organic chemicals consisting of various methylated phenols. EFS contain an extraordinarily high content of tocopherols, which may contribute to scavenge free radicals and antioxidant effects. Li et al. isolated and identified three tocopherols, *α*-tocopherol (**31**), β-tocopherol (**32**), and δ-tocopherol (**33**), from EFS [38]. Rowet al. obtained two new tocopherol trimers, ferotocotrimer C (**34**) and D (**35**), and two known tocopherol trimers, IVb (**36**) and IVa (**37**) from the seeds of *E. ferox* [42]. In addition, two new tocopherol polymers, the chroman-type dimer ferotocodimer A (**38**) and the spiro-type trimer ferotocotrimer E (**34**), were isolated from EFS [48]. Their structures were determined on the basis of spectroscopic data, especially 1D and 2D NMR analysis (**Figure 6**).

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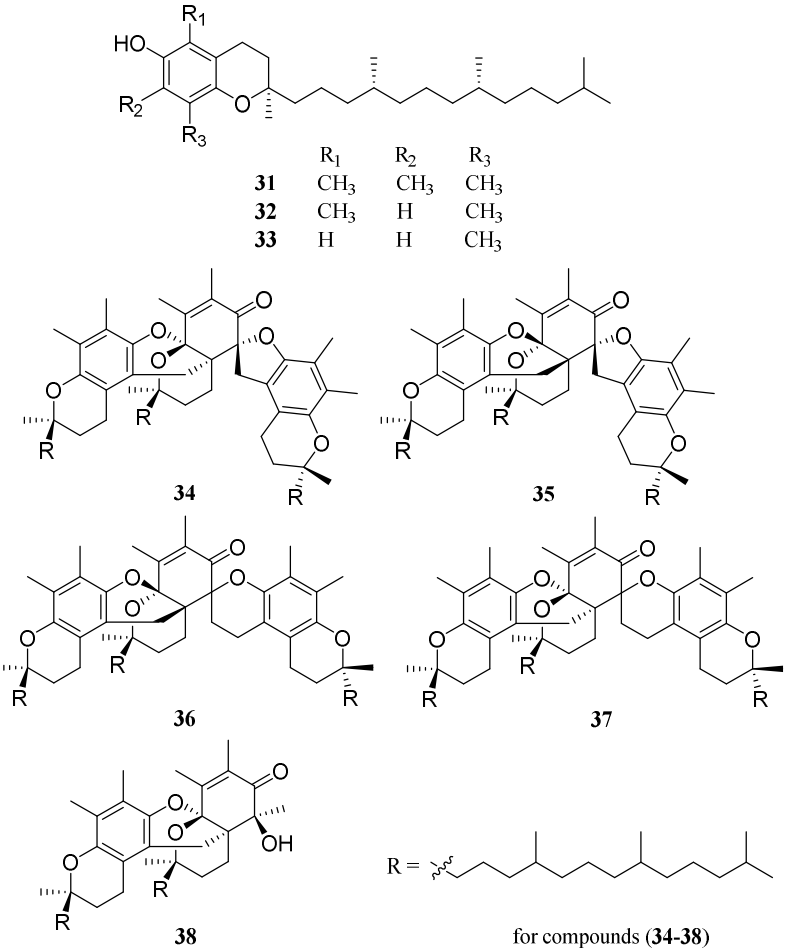

Figure 6. Chemical structures of tocopherols of *E. ferox*.

5.7. Lignans

In order to obtain the antioxidant compounds which may be beneficial for the treatment of proteinuria of diabetic nephropathy, Song et al. investigated the components in the ethyl acetate extract of EFS, 3 new sesquineolignans, named euryalins A-C (**39**-**41**), 2 neolignans, named rel-(2*α*,3β)-7-*O*-methylcedrusin (**42**) and syringylglycerol-8-*O*-4-(sinapyl alcohol) ether (**43**), and 3 furofuran-type lignans, named (1*R*,2*R*,5*R*,6*S*)2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**44**), (+)-syringaresinol (**45**), and buddlenol E (**46**), were obtained [39]. In addition, another 2 antioxidative lignans, (+)-Isolariciresinol 9'-*O*-glucoside (**47**) and 3-(4-hydroxy-3-methoxybenzyl)-4-[(7'R),5'-dihydroxy-3'-methoxybenzyl]tetrahydrofuran (**48**), were also isolated from EFS [37, 38]. The isolated lignans are shown in **Table 2**, and the corresponding structures are shown in **Figure 7**.

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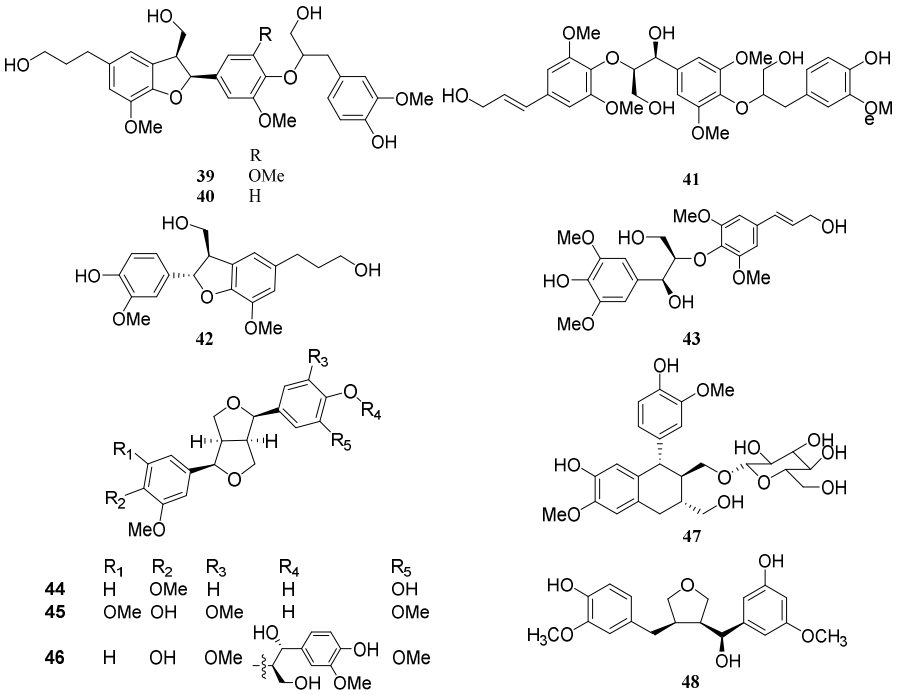

Figure 7. Chemical structures of lignans of *E. ferox*.

5.8. Essential Oil

A total of 37 components were identified by gas chromatography-mass spectroscopy in the essential oil isolated by steam distillation in EFS. The main constituents of the oil were butylated hydroxytoluene (**49**) (38.7 %), palmitic acid (**50**) (11.0 %), linoleic acid (**51**) (9.0 %), and hexanoic acid (**53**) (3.9 %) [46]. The structures of essential oils (**49**-**83**) are shown in **Figure 8**.

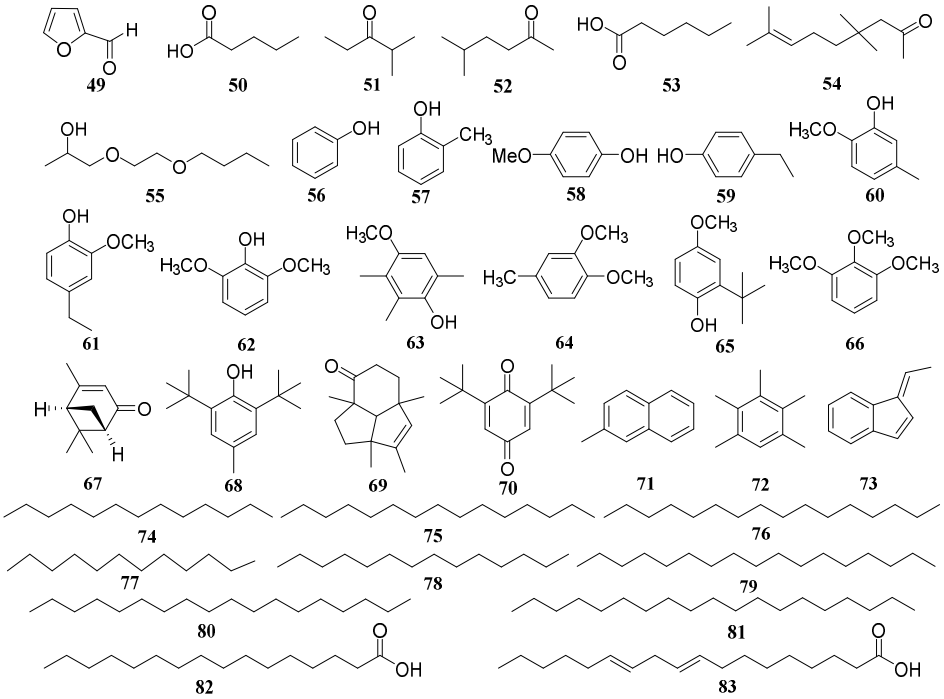

Figure 8. Chemical structures of essential oils of *E. ferox*.

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5.9. Others

Other phytochemicals reported in *E. ferox* include esters (**84, 85**), ketones (**86, 87**), aldehydes (**88-90**), alcohols (**91**, **92**), phenols (**93**, **94**, **95**), and sulfoxide (**96**). In addition, 16 nucleosides and nucleobases (**97-112**) were simultaneously determined by HPLC-ESI-TQ-MS/MS, the contents of them in 26 batch samples were quantified with standards [49]. An ultrasound assisted technique was established for the extraction of anthocyanins from the waste leaves of *E. ferox*, and the yield of anthocyanins was 2.82±0.03 mg/g. The HPLC-QTOF-MS/MS identified 19 anthocyanins in the extract of *E. ferox* leaves [26] (**Figure 9**).

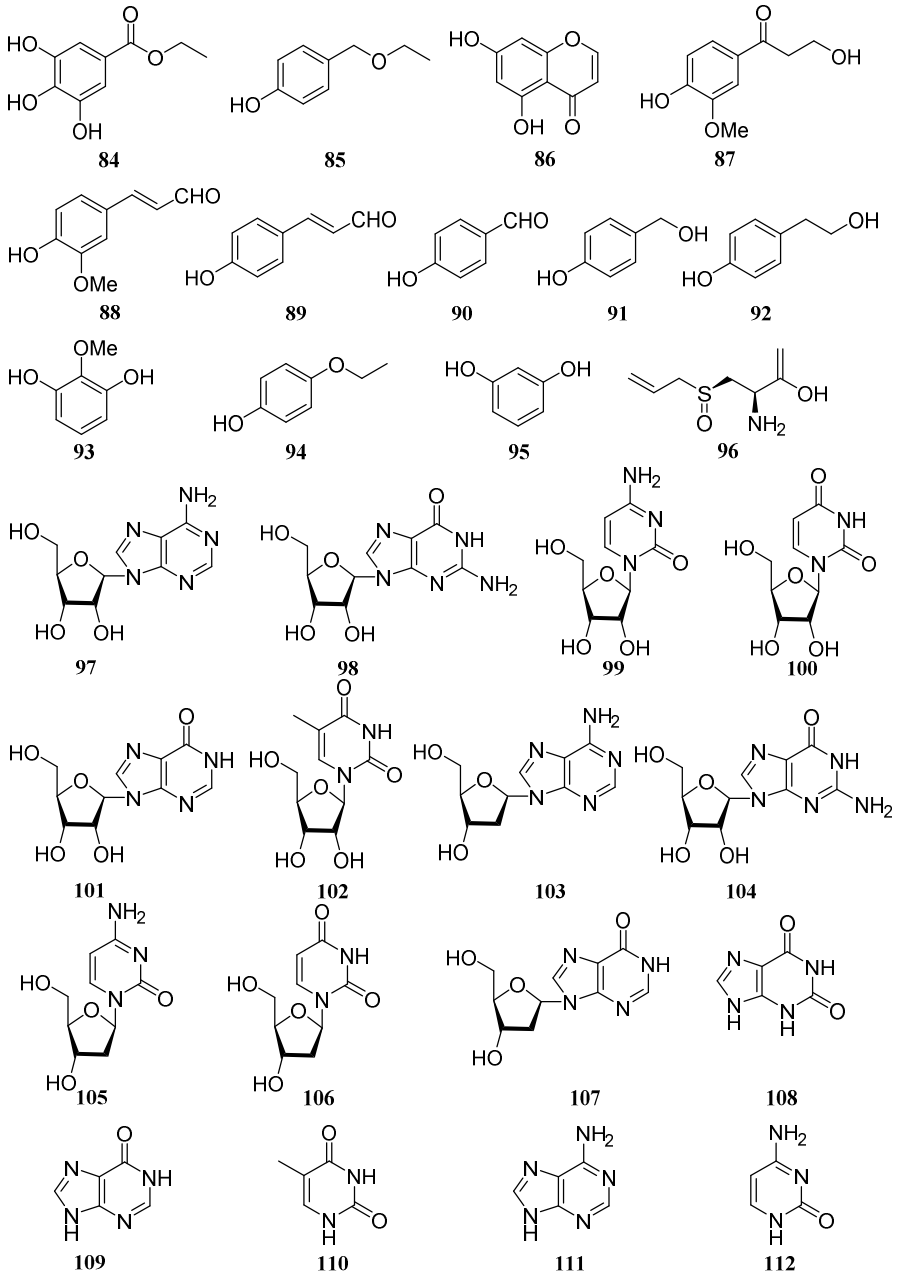

Figure 9. Chemical structures of other compounds of *E. ferox*.

6. Pharmacological activities

Various in vitro and in vivo studies have indicated that *E. ferox*-derived extracts and phytoconstituents exhibit superior antioxidant and antidiabetic effects. In addition, the anti-tumor, anti-hyperlipidaemic, antibacterial, anti-inflammatory, antimelanogenic, antiaging, antifatigue, cardioprotective, and hepatoprotective activities were also reported. The pharmacological properties of *E. ferox* were summarized in **Table 3** and **Figure 10**.

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Table 3 Pharmacological activities of *E. ferox*-derived extracts and phytoconstituents.

Activity	Compound/extract	Plant part	Animals/cell lines	Doses	Effects	Ref
Antioxidation and anti-inflammation	Methanol extract	Seeds	DPPH scavenging assays V79-4 cell line RAW 264.7 cell line	For antioxidation, 0.8-100 μg/ml For antiinflammation, 100-400 μg/ml	DPPH radical scavenging activity (IC_{50} 22.95±0.25 μg/ml or 5.6 μg/ml); iNOS, Cox-2, NO inhibition (300-400 μg/ml); Inhibition of lipid peroxidation (IC_{50} 20.5 μg/ml)	[19, 20]
	Ethanol extract Compounds **40**, **42**-**46**	Seeds	DPPH scavenging assays ROS model: glucose treated mesangial cells	For DPPH: 2-1000 μg/mL For ROS: 1, 10 μM	DPPH (SC50) of ethanol extract, compounds **40**-**43**, **45** were 103.1 μg/ml, 6.8, 10.4, 10.2, and 12.9 μM, respectively; Compounds **40**, **42**, **44**-**46** showed ROS inhibition at 10 μM	[39]
	Aqueous extracts	Seeds	DPPH and ABTS scavenging activity H_2O_2-induced human skin fibroblast oxidative stress	/	DPPH and ABTS scavenging; Increased expression of SOD, CAT, and GSH-Px	[21]
	Phenolic extracts	Seed shells	DPPH scavenging assays	0.1-1.0 mg/mL	DPPH scavenging activity is similar to vitamin C and Trolox at 1 mg/mL	[36]
	Phenolic extracts	Seed shells	DPPH and Hydroxyl scavenging assays D-galactose-induced aging Kunming mice	In vitro: 0.01-2 mg/mL In vivo: 100, 200, 400 mg/kg p.o. once daily for 32 days	Strong DPPH and Hydroxyl scavenging activity; Increases the SOD, CAT, GSH-Px activities and decreases MDA content in the liver and kidneys	[18]
	Anthocyanins extraction	Leaves	DPPH and ABTS scavenging activity	/	DPPH and ABTS scavenging IC_{50} were 74.00±3.63 μg/mL and 5.77±0.28 μg/mL, respectively	[26]
	Ethyl acetate, ethanol, or 50% ethanol extract	Seed shells	DPPH scavenging assays Lipid peroxidation	5-200 μg/mL	DPPH radical scavenging activity (IC_{50} 29.4±1.34, 28.3±1.21, 27.60±1.02 μg/mL, respectively); Inhibition of lipid peroxidation (IC_{50} 43.86±1.32, 30.44±1.15, 36.42±1.43, respectively)	[54]

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	Cell wall polysaccharides	Petioles and pedicels	DPPH and ABTS scavenging activity H_2O_2-induced injury on HUVEC and VSMC	For DPPH and ABTS: up to 6.5 mg/mL For cell model: 60 and 200 μg/mL	Around 80% DPPH scavenging activity at 3.25 mg/mL, and 100% ABTS scavenging activity at 1.625 mg/mL; Reduced MDA levels, and increased T-AOC, SOD and CAT activities in H_2O_2-injuryed VSMC and HUVEC cells.	[55]
	Essential Oil	Seeds	DPPH and ABTS scavenging activity	0.5, 1, 2, 4 and 8 μg/mL	DPPH and ABTS scavenging IC_{50} were 6.27 ± 0.31 and 2.19 ± 0.61 μg/ml, respectively	[46]
Antidiabetic and hypoglycemic effects	70% ethanol extract	Seeds	Streptozotocin-induced diabetic Wistar rats	100, 200, 300, 400 mg/kg, p.o. for 45 days	Significantly decreased the blood glucose level, increased plasma insulin level, restored hepatic gluconeogenic enzymes activities; increased activities of SOD, CAT, GPx, and GSH	[15]
	70% methanol extract	Germinated seeds	Streptozotocin-induced diabetic ICR mice	100, 200, 400 mg/kg, p.o. for 4 weeks	Improved hyperglycemia, abnormal lipid metabolism, and renal tissue lesions; Decreased kidney microalbuminuria, blood urea nitrogen, serum creatinine, MDA and GSH; Increased activity of CAT, SOD, serum total antioxidant capacity; Regulating the Keap1/Nrf2/HO-1 and AMPK/mTOR pathways.	[56]
	Polysaccharide (EFSP-1)	Seeds	Dexamethasone-induced HepG2 and 3T3-L1 preadipocyte cells	Incubation with 25, 100, 400 μg/mL EFSP-1 for 24 h	Increasing glucose consumption by up-regulating the expression of GLUT-4 via activating PI3K/Akt signal pathway in insulin resistance cells	[32]
	2β-hydroxybetulinic acid 3β-caprylate (HBAC)	Seeds	Streptozotocin-induced diabetic Wistar rats	20, 40, 60 mg/kg, p.o. for 45 days	Exhibited free radical scavenging property, pancreas and hepatoprotective effect;	[44]

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2β-hydroxybetulinic acid 3β-oleiate (HBAO)	Seeds	Streptozotocin-induced diabetic Wistar rats	20, 40, 60 mg/kg, p.o. for 45 days	Stimulating insulin release; Improved the glycemic control and lipid profile Alleviating glycemic homeostasis and oxidative stress, normalized plasma glucose, glycosylated hemoglobin (HbA1c), hepatic gluconeogenic enzymes, plasma insulin, ameliorating pancreatic β-cell, hepatic and renal histology and β-cell functions, improving dyslipidemia and antioxidant enzymes	[45]
Ethanol extract	Seed shells	α-amylase and α-glucosidase	20-100 μg/mL	The inhibitory effects of *E. ferox* seed shell extract (EFSSE) on α-amylase and α-glucosidase in terms of IC_{50} was 62.95 and 52.06 μg/mL, respectively.	[57]
Triterpenoid-rich 75% ethanol extracts	Seed shells	Streptozotocin-induced diabetic mice	200, 400, 600 mg/kg, p.o. for 4 weeks	Regulating glucose metabolism and body weight; Decreased cholesterol, LDL and triglycerides levels, and increased HDL	[53]
Triterpenoid-rich 75% ethanol extracts	Seed shells	Streptozotocin-induced diabetic Kunming mice	200, 300, 400, 500±2 mg/L in drinking water for 4 weeks	Restored glucose metabolism and body weight; Recovered Islet morphology; Reduced PTP1B protein and increased insulin receptor IRS-1 protein	[58]
Network pharmacology method	/	The TCMSP, SymMap V2, CTD, DisGeNET, and GeneCards databases were searched for ES components, targets, and DKD targets	/	The main components are oleic acid and vitamin E, targeting the proteins PPARA, LPL, FABP1, MAPK1 to regulate TNF, apoptosis, and MAPK.	[59]
Polysaccharides	Petioles and pedicels	Alloxan-induced hyperglycemic ICR mice	100, 200, 400 mg/kg, p.o. for 28 days	High dose of EFPP reverse alloxan-induced body weight loss, reduce blood glucose level, enhance serum insulin level, improve oral	[60]

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					glucose tolerance, increase hepatic glycogen content and GCK activity; increase SOD, CAT and GSH-Px activities and decrease MDA contents in liver and kidney	
Hepatoprotective and cardioprotective activities	Ethanol extract	Seed shells	High fat diet induced ICR mice	15 and 30 mg/kg, p.o. for 28 days	Reduced body weight, lipids deposition in the liver and blood lipids, decreased MDA content and increased SOD activity; IRs-1 activation and CYP2E1 inhibition	[13]
	Ethanol extract	Seeds	Ischemia and reperfusion in vitro model; Chronic ischemic reperfusion injury in vivo model	25, 125 or 250 μg/ml for in vitro; 50 and 500 mg/kg, p.o. for 21 days	Improved post-ischemic ventricular function and reduced myocardial infarct size; increased expression of TRP32 and thioredoxin proteins; ROS scavenging activities	[14]
Anticancer	Ethanol extract	Seeds	In vitro: A549 Human Caucasian Lung Carcinoma cancer cells In vivo: Balb/c nu/nu mice	In vitro: 50-150μg/mL In vivo: 100 mg/kg/day for 28 days	In vitro: promoting A549 apoptosis via inhibition of the Akt protein and activation of the p53 protein; In vivo: activating p53 and suppressing the tumor growth	[16]
	Ethanol extract	Seed shells	Human Gastric Cancer SGC7901 cells and Human Hepatoma HepG2 cells	50-800 μg/mL	Inhibitory effect on the proliferation of SGC7901 cells and HepG2 cells were 92.63% and 72.40%, respectively	[61]
	Ethyl acetate fraction	Seeds	Melan-a cells	3-30 μg/mL	Inhibition of cellular tyrosinase and melanin synthesis	[23]
	Resorcinol	Seeds	B16F10 melanoma cells	/	Inhibition of melanin synthesis in B16F10 melanoma cells with an IC50 492.8 μM	[37]

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Cytotoxicity	Ferocerebrosides A and B	Seeds	Brine shrimp lethality bioassay	62.5, 125, 250, 500, and 1000 μg/mL for 24 h	Ferocerebrosides A and B showed marginal toxicity against brine shrimp with LC50 values of 0.17 and 0.20 mM, respectively	[42]
	Polysaccharide fraction (EFSP-1)	Seeds	3T3-L1 preadipocyte cells and HepG2 cells	25, 50, 100, 200, 400 μg/mL for 48 h	No obvious influence to cells at 100–400 μg/mL	[32]
	Hexane, diethyl ether, ethyl aetate extract	Seeds	Glutamate-induced cytotoxicity in hybridoma N18RE-105 cells	10 μg/mL for 24 h	Dose-dependent protection against neuronal cell death induced by 20 mM glutamate	[62]
Anti-fatigue	Phenolics extract	Seed shells	Exhaustive swimming test	100, 200, 400 mg/kg p.o. once daily for 32 days	The average exhaustive swimming time was obviously prolonged in all three doses	[18]
Anti-depressant	Petroleum ether fraction	Seeds	Chronically unpredictable mild stress (CUMS) mouse model	0.1, 0.15 g/kg p.o. once daily for 14 days	Upregulation of AMPK and ULK1, attenuated depressive behavior via AMPK-ULK1 pathway mediated autophagy	[22]

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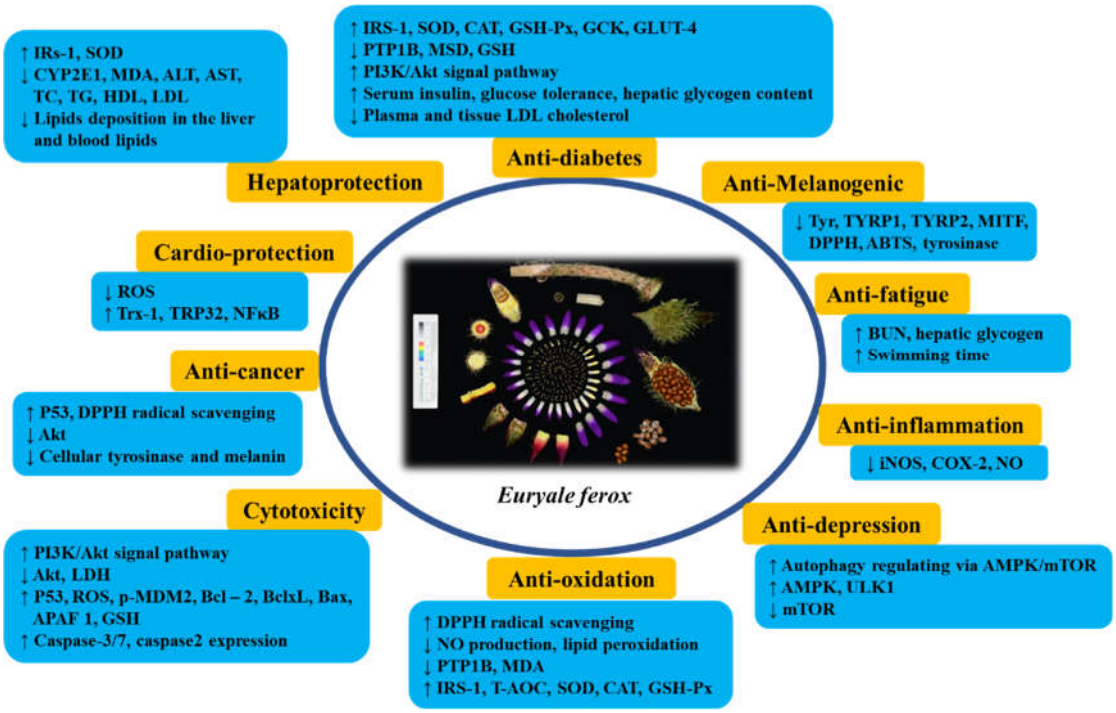

Figure 10. The summarized pharmacological mechanism and effects of *E. ferox*.

6.1. Antioxidant and anti-inflammatory activity

The evaluation of antioxidant activity can be performed in three main ways including directly 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reactive oxygen species (ROS) scavenge, the activation of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and improvement of somatic cellular integrity. The methanol, ethanol, and aqueous extracts of EFS showed DPPH scavenging effects, while the methanol extract showed anti-inflammatory activity in RAW 264.7 cell line [19-21, 39].

EFS methanol extracts exert high levels of DPPH radical scavenging activity, lipid peroxidation inhibition, protection of H_2O_2-induced apoptosis and the antioxidant enzymes activity enhancement. Among various fractionated samples of *E. ferox*, the ethyl acetate and butanol fractions exhibited relatively high antioxidative activity [20]. The essential oil from the EFS exhibited strong DPPH and ABTS scavenging activity, the IC_{50} of which were 6.27 ± 0.31 and 2.19 ± 0.61 μg/ml, respectively [46]. Fermentation of *E. ferox* with *Lactobacillus curvatus* increases the content of the various bioactive components including smaller molecular weights of polysaccharides and polypeptides, enhances its antioxidant capacity and attenuates oxidative stress-induced human skin fibroblast apoptosis and senescence [21]. In addition, the phenolic extracts from the *E. ferox* seed shells and anthocyanins from the *E. ferox* leaves showed DPPH and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging effects [18, 26, 36].

Cell wall polysaccharides (EFPP) were isolated from the petioles and pedicels of *E. ferox* using DEAE-52 column, and four major fractions (EFPP-1, EFPP-2, EFPP-3 and EFPP-4) were obtained. The crude EFPP and EFPP-4 could effective against H_2O_2-induced injury on HUVEC and VSMC through enhancement of T-AOC, SOD and CAT activities and decrease of MDA content [55].

The anti-oxidative activities vary from different parts of *E. ferox*, while the seed extracts showed better effects than seed shells, leaves, and petioles and pedicels from the aforementioned reports. Further studies should aim to purify and characterize the active phytoconstituents from the antioxidative extracts.

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6.2. Antidiabetic and hypoglycemic activity

The *E. ferox* ethanol extract protected β-cells against ROS-mediated damage by increasing the expression of antioxidant enzymes and reducing hyperglycemia, possibly due to the release of insulin from residual and recovered β-cells in the pancreas of streptozotocin-induced diabetic rats [15]. Another study indicated that germinated EFS extract contained more gentisic acid, caffeic acid, and other 27 effective polyphenols than EFS, corresponding to the higher improved antioxidant and renal indexes, and a more stable effect in regulating the AMPK/mTOR and Keap1/Nrf2/HO-1 signaling pathways, leading to the more attenuated antidiabetic effects [56]. A polysaccharide obtained from EFS, EFSP-1, could increase glucose consumption by up-regulating the expression of GLUT-4 *via* activating PI3K/Akt signal pathway in insulin resistance HepG2 and 3T3-L1 cells [32]. The antidiabetic activities of two triterpenoids in *E. ferox* were investigated in streptozotocin-induced Wistar rats over a four-week period. After 45 days gavage of 2β-hydroxybetulinic acid 3β-caprylate (HBAC) and 2β-hydroxybetulinic acid 3β-oleiate (HBAO) in diabetic mice, the plasma glucose and insulin were normalized, pancreatic β-cell, histological architecture of pancreas, kidney, and liver were restored, as well as the endogenous antioxidant enzymes [44, 45]. The aforementioned studies suggest that extract of EFS could be an important source of natural antioxidants with hypoglycaemic and hypolipidaemic effects, and could be used as a food additive or functional food in the future.

Another study investigated the extract of Gorgon fruit as a food additive and found that *E. ferox* shell extract (EFSSE) had a significant effect on the in vitro digestibility of bread starch, and that EFSSE (2%) fortified bread and exhibited a strong glycemic index inhibition. In addition, the IC_{50} of EFSSE on α-amylase and α-glucosidase inhibitory effect was 62.95 and 52.06 μg/mL, respectively [57]. The hypoglycemic and hypolipidemic effects of triterpenoid-rich 75% ethanol extracts of *E. ferox* shell were investigated in streptozotocin-induced diabetic mice. Gavage of 400 and 600 mg/kg *E. ferox* shell extract for 4 weeks significantly restored the body weight, blood glucose, and insulin resistance [53]. Triterpenoid-rich *E. ferox* shell extract in drinking water (500 mg/L) for 4 weeks significantly attenuated streptozotocin-induced high blood glucose, pancreas injury, higher tyrosine phosphatase-1B level and low insulin receptor substrate expression [58]. Therefore, *E. ferox* shell extract can be used as a therapeutic ingredient for diabetes induced by insulin resistance.

Crude polysaccharides (EFPP) were prepared from the petioles and pedicels of *E. ferox*, which had a total carbohydrate of 65.72 ± 2.81%, the monosaccharide compositions were Man, GlcA, Rha, Glc, Gal and Ara at a molar ratio of 0.12:0.01:9.57:0.41:1.00:0.24. After oral administration with EFPP (400 mg/kg) for 28 days, the activities of CAT, SOD and GSH-Px and MDA contents in kidney and liver of alloxan-induced mice were significantly ameliorated, as well as the damaged pancreas, kidney and liver tissues. The blood glucose level was reduced and the serum insulin level was increased remarkably [60].

Currently, network pharmacology as an emerging discipline has been gradually applied to the mechanistic study of phytopharmaceuticals. This method is suitable for multi-component and multi-target studies by using the database of ingredients, targets and genes to elucidate the complex mechanism of action of a drug in a holistic way. Some investigations have preliminarily elucidated the anti-diabetic mechanism of action of *E. ferox* based on network pharmacology and molecular docking. Twenty-four components of *E. ferox* and 72 targets were identified, of which 9 (FABP1, JUN, LPL, PPARA, TP53, TGFB1, IL1A, MAPK1, CTNNB1) are clinically relevant and mainly regulated by transcription factors such as HNF4A and PPARG. The main components are oleic acid, which targets the proteins encoded by PPARA, LPL and FABP1, and vitamin E, which binds to the proteins encoded by MAPK1 and TGFB1[59].

In conclusion, *E. ferox* can be used to treat diabetes mainly through anti-inflammatory, reducing pancreatic β-cell damage and apoptosis, promoting glucose absorption and utilization, and improving insulin resistance and complications. Although noteworthy antidiabetic properties have been attributed to *E. ferox* polysaccharides or triterpenoids, the homopolysaccharide has not been identified and if there are any other phytoconstituents responsible for this activity remains to be

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elucidated. Meanwhile, further clinical validation of the above findings is still needed in conjunction with experiments.

6.3. Hepatoprotective and cardioprotective activity

Oral administration of the *E. ferox* seed coat ethanol extract (EFSCE) to high-fat diet (HFD)-induced ICR mice at doses of 15 and 30 mg/kg for 4 weeks resulted in a significant reduction in body weight, lipid deposition in the liver and blood lipids. EFSCE also prevented excessive production of MDA and enhanced SOD activity to counteract oxidative stress. In addition, EFSCE was effective in reducing alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the HFD-induced mice. EFSCE is used as a biologically active natural product for the treatment of HFD-induced NAFLD by modulating IRs-1 and CYP2E1 to eliminate lipid accumulation and oxidative stress [13].

Another study investigated if *E. ferox* seeds could reduce myocardial ischemic reperfusion injury. The isolated rat hearts ischemia and reperfusion acute model was constructed to evaluate the cardioprotective effect of *E. ferox* extract (25, 125 or 250 μg/ml), 125 or 250 μg/ml *E. ferox* extract treatment significantly enhanced aortic flow and reduced the infract size. *E. ferox* (250 and 500 mg/kg/day) oral administration for 21 days improved post-ischemic ventricular function and reduced myocardial infarct size in a chronic ischemic reperfusion model. Two cardioprotective proteins, TRP32 and thioredoxin, were significantly increased. Taken together, this study demonstrated the cardioprotective properties of Makhana and the effects may be related to its upregulation of TRP32 and Trx-1 proteins and ROS scavenge activities [14].

6.4. Cytotoxic and anticancer activity

The apoptotic effects of EFS ethanol extract (ESE) in A549 lung cancer cells were investigated, ESE induces apoptosis via the regulation of mitochondrial outer membrane potential and generation of ROS. ESE-induced A549 apoptosis is in a p53-dependent manner, in addition, ESE suppressed tumor growth in Balb/c-nu mice bearing A549 xenografts and activated p53 protein [16]. *E. ferox* seed shell extracts (200 μg/mL) showed an inhibitory effect on SGC7901 and HepG2 cells proliferation, with the inhibition rate being 92.63% and 72.40%, respectively. *E. ferox* seed shell extracts (200-800 μg/mL) arrest SGC7901 cells in the G0/G1 phase, and 50-200 μg/mL *E. ferox* seed shell extracts arrest HepG2 cells in the S phase. While, the cell mitochondrial membrane potential was significantly reduced and the intracellular calcium influx was increased [61]. Treatment of melan-a cells with 30 μg/mL EFS ethyl acetate fraction produced a strong inhibition of cellular tyrosinase and melanin synthesis, and the lysosomal degradation of tyrosinase was involved in melanogenesis inhibition [23]. Resorcinol (**95**) inhibited melanin synthesis in B16F10 melanoma cells with an IC50 value of 492.8 μM [37].

Two cerebrosides, ferocerebrosides A and B, were isolated from the methanol extract of EFS, and they showed marginal toxicity against brine shrimp with LC50 values of 0.17 and 0.20 mM, respectively [42]. The toxicity study of a new glucan EFSP-1, obtained from EFS, was performed on HepG2 and 3T3-L1 cells, and no obvious toxic was observed at doses among 100 and 400 μg/mL [32]. Neuroprotective effect of EFS subfractions against glutamate-induced cytotoxicity in hybridoma cells N18-RE-105 was investigated. The EFS ethanolic extract showed a dose-dependent protective effect against 20 mM glutamate-induced neuronal cell death. EFS ethanolic extract was subfractionated with hexane, diethyl ether, and ethyl acetate, the hexane fraction showed strongest neuroprotective effect against glutamate-induced N18-RE-105 cells. The results suggest that EFS can be used as chemotherapeutic agents in the treatment of neurological disorders [62].

6.5. Antifatigue activity

An exertional swimming test was performed to evaluate the anti-fatigue effect. The phenolic extracts of *E. ferox* can prolong the average duration of exertional swimming, the expression of BUN

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was significantly reduced, while hepatic glycogen content was dramatically increased. In addition, three main phenolic compounds in the extract were identified as 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-chroman-4-one, naringenin, and buddlenol E [18].

Studies have shown that *E. ferox* is a potential and readily available source of natural antioxidants and has the potential to be a new functional anti-fatigue food or drug. In the future, studies on the chemical composition and safety evaluation of phenolic extract need to be continued with a view to provide valuable information for the novel functional food development.

6.6. Anti-depressant activity

The potential antidepressant effects of EFS petroleum ether fraction (ES-PE) were investigated in a mouse model of chronic unpredictable mild stress (CUMS). Deficits in the open field test, sucrose preference test, tail suspension test and forced swimming test were observed in mice following CUMS and were reversed following ES-PE administration. ES-PE significantly up-regulated phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and mammalian autophagy initiating kinase (ULK1) at Ser317, the ration of p-mTOR/mTOR was suppressed by ES-PE treatment. In addition, ES-PE treatment significantly attenuated Compound C, an inhibitor of AMPK, induced autophagy suppression. GC-MS analysis revealed high levels of vitamin E acetate in ES-PE, suggesting the potential role of VE in the antidepressant effect of ES-PE [22]. Further studies are needed to explore the antidepressant mechanism of ES-PE, in addition to autophagy, as well as other potential phytochemicals.

7. Toxicity

The non-toxic characteristic of EFS was clearly stated thousands of years ago in the Shennong's Herbal Classic [5]. According to Chinese Pharmacopoeia, the medicinal dosage of EFS is generally 9–15 g per day [13]. If it is excessive consumed, it may lead to gastrointestinal overload, as EFS contains a lot of starch, protein and other ingredients that have a solid and astringent effect [34, 57]. The "Suixiju Dietary Recipes" records the contraindications of EFS as "EFS is not recommended in the following conditions, including before and after cold affection, malaria, dysentery and hemorrhoids, red urine and constipation, transport failure of spleen, and postpartum period". Nevertheless, the monitoring of EFS adverse reactions should be further strengthened in order to improve its safety in clinical application.

8. Conclusions and perspectives

The current study summarized the investigations of *E. ferox* in terms of traditional uses, phytochemistry, pharmacological effects, and toxicity in recent decades. It is expected to provide a preliminary basis for future research on *E. ferox* and to provide a reference for further studies on the biological activities and clinical applications.

Firstly, *E. ferox* contains a complex and diverse chemical constituent, including triterpenes, sterols, flavonoids, phenylpropanoids, essential oils, organic acids and polysaccharides. So far, more than 100 compounds have been isolated and identified. However, although many chemical components have been elucidated, a few of them have been validated for their biological activity. There is a lack of in-depth studies on the mechanisms of physiological activity of polysaccharides. The homosaccharide, structural information, monosaccharide composition and content need to be further investigated.

Secondly, the anti-diabetes, gastrointestinal diseases and even anti-cancer effects of *E. ferox* has been greatly explored. However, the understanding of its mechanisms and pathways of action remains ambiguous and is mostly based on its anti-oxidant effects. In addition, rarely no studies addressed the toxicities of *E. ferox*, and the pharmacokinetics and drug interactions of *E. ferox* in vivo remain unknown.

Further research and development are needed in the following aspects. On the one hand, the isolation, purification, and identification of chemical components should be continued, with emphasis on the biological activity and structure-activity relationships. On the other hand, to obtain intuitive evaluations via animal and clinical experiments, new techniques and methods such as molecular biology, cell biology and histology should be combined to further explore the intrinsic pharmacological mechanisms.

As a medicinal food ingredient with rich nutritional value and various health functions, *E. ferox* have good prospects for market development. The design of *E. ferox* special functional food and the utilization of its derived waste material might be the future directions.

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