The phytochemical, biological, and medicinal attributes of phytoecdysteroids: An updated review

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\textbf{Abstract} The phytoecdysteroids (PEs) comprise a large group of biologically-active plant steroids, which have structures similar to those of insect-molting hormones. PEs are distributed in plants as secondary metabolites that offer protection against phytophagus (plant-eating) insects. When insects consume the plants containing these chemicals, they promptly molt and undergo metabolic destruction; the insects eventually die. Chemically, ecdysteroids are a group of polyhydroxylated ketosteroids that are structurally similar to androgens. The carbon skeleton of ecdysteroids is termed as cyclopentanoperhydro-phenanthrene with a $\beta$-side chain at carbon-17. The essential characteristics of ecdysteroids are a cis-(5$\beta$-H) junction of rings A and B, a 7-en-6-one chromophore, and a trans-(14$\alpha$-OH) junction of rings C and D. Plants only synthesize PEs from mevalonic acid in the mevalonate pathway of the plant cell using acetyl-CoA as a precursor; the most common PE is 20-hydroxyecdysone. So far, over 400 PEs have been identified and reported, and a compilation of 166 PEs originating from 1998 has been previously reviewed. In the present review, we have summarized 212 new PEs reported between 1999 and 2019. We have also critically analyzed the biological, pharmacological, and medicinal properties of PEs to understand the full impact of these phytoconstituents in health and disease.

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Phytoecdysteroids in health and disease

1. Introduction

Phytoecdysteroids (PEs) are a class of biologically-active chemicals thatplants synthesize for defense against phytophagous (plant-eating) insects. The name for ecdysteroids originate from the word “ecdysis”, which in turn is derived from the Greek word “ecdysis”, meaning shedded outer skin. Ecdysteroids were originally considered a class of steroid hormones that control the processes of insect moulting and metamorphosis\(^1\). The first ecdysteroid, named edcysone, was isolated by Butenandt and Karlson\(^2\) in 1954 from the silkworm pupae; its structure was elucidated in 1965 by Huber and Hoppe\(^3\) using X-ray crystallography. Arthropod steroid hormones also known as zoecdysteroids were shown to regulate the development of arthropods and other invertebrates as well. The metabolism and pharmacological effects of such ecdysteroids has drawn considerable attention in mammalian systems\(^4\). Isolation of several members of this class from different plant sources indicate that these compounds not only control the moulting and metamorphoses of invertebrates, but also have control of other biological functions in plants and invertebrates\(^5\). The ecdysteroids derived from plants are called phytoecdysteroids; ecdysteroids derived from animals are known as zoecdysteroids. Several common ecdysteroids, such as edcysone (E), 20-hydroxyecdysteroid (20-HE), makisterone A, and ajugasterone C, can be found in both plants as well as animals\(^6\). PEs are spread throughout the plant kingdom; however, a survey indicates that only \(\sim 6\%\) of all plants contain detectable levels of ecdysteroids\(^7\). A series of evidence suggests that PEs do not accumulate in the majority of plant species as the expression of the biosynthetic pathways in plants is downregulated\(^8\). In plants, they provide chemical defence against certain herbivorous insects through their allelochemical properties; one of such includes taste receptors which emithormonal disruptions and signal toxicity to insects and other invertebrates\(^9\). These chemicals are also able to alter and switch genes around in plants through DNA binding\(^10,11\). For further study in this field, several editors, book chapters, conference abstracts, and unpublished results were not incorporated. Only articles written in English were included in this review. Major keywords used include: phytoecdysteroids, phytoecdysones, ecdysteroids, edcysones, secondary metabolites, phytoinsecticides, antioxidant, anti-

dietary supplements containing ecdysteroids, especially 20-HE, are being marketed in the United States.

PEs, biosynthetically derived from cholesterol or other plant sterols, have multiple physiological roles in plants, and have a broad spectrum of pharmacological and medicinal properties in mammals. The major properties of PEs include hepatoprotective, hypoglycaemic, and anabolic effects on skeletal muscle\(^12\). PEs have been utilized by sportsmen and body-builders for improving physical performance, and for enhancing stress resistance by promoting vitality\(^13\). Different PEs could show binding interactions with human nuclear receptors\(^14\). The PE derivatives displayed hormonal effects on invertebrates and exerted several favorable, non-hormonal, biological effects on mammals\(^15\). The phytochemical properties of PEs and prospective bioactivities are novel in nature with respect to their abundance and application. Yet, a paucity of comprehensive records exists which nessecciates a need to summarize the current state of knowledge on biosynthesis, distribution, biological, and physiological properties of PEs and pharmacological impacts on human physiology and metabolism in normal and diseased conditions. However, the cumulative information remains limited to the review of 166 PEs reported by Baltaev in 1998\(^24\). Thus, there is a need for updated and comprehensive information on ecdysteroids. In the past two decades, numerous research studies have reported diverse chemical and biosynthetic characteristics of PEs and their physiological roles in plants as well as their pharmacological and biomedicinal properties. In view of these advances, the present review summarizes the current knowledge on the biosynthesis, distribution, biological importance, and pharmacological applications of PEs. We have summarized 212 new phytoecdysteroids reported from 18 plant families since 1999. These PE derivatives have been critically analyzed for their biological, pharmacological, and medicinal properties in order to understand the impact of these phytoconstituents in health and disease.

2. Methodology for literature search

The majority of the literature search was conducted electronically on multiple databases, such as Scifinder (http://cas.org/products/scifinder/index.html). Additional information was collected from reliable and authentic databases such as PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Science Direct, Scopus, Web of Science, Google Scholar, and the ecdysteroids electronic database (http://ecdysbase.org). For this systematic review, we have followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines\(^25\). Appropriate and high-quality publications (1999–2020) were collected. Letters from editors, book chapters, conference abstracts, and unpublished results were not incorporated. Only articles written in English were included in this review. Major keywords used include: phytoecdysteroids, phytoecdysones, ecdysteroids, edcysones, secondary metabolites, phytoinsecticides, antioxidant, anti-

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inflammatory, antimicrobial, antidiabetic, anticancer properties, in vitro and in vivo studies.

3. Chemistry of phytoecdysteroids

The carbon skeleton of ecdysteroids is termed cyclopentanoperhydro-phenanthrene and has a β-side chain at carbon-17. The essential characteristics of ecdysteroids are their composition of a cis-(5β-H) junction of rings A and B, a 7-en-6-one chromophore, and a trans-(14α-OH) junction of rings C and D. The sterol structure is modified to produce ecdysteroids; the trans A/B ring juncture in sterols is converted to a cis A/B ring juncture in the ecdysteroids. Chemically, these are C27, C28, or C29 polyhydroxy steroids that have a 14α-hydroxy-7-en-6-one chromophore and A/B-cis ring fusion. 20-HE (1) was identified from different varieties of arthropods and is now considered a major biologically-active ecdysteroid in arthropods. Its atom numbering is depicted in Fig. 1.

Chemically, ecdysteroids are polar steroids in nature; their solubility is almost identical to that of a sugar molecule. As a result, they are soluble in aqueous mediums and are lipophilic. The mammalian steroid hormones have more variable structures and they generally lack the polyhydroxylated side chain characteristic of ecdysteroids; accordingly, they are quite nonpolar. Unlike invertebrates, which are incapable of synthesizing ecdysteroids and must consume dietary phytosterols that transform into ecdysteroids, plants can completely synthesize ecdysteroids from mevalonic acid and cholesterol. So far, more than 200 PEs, mostly from genus Ajuga, Podocarpus, Polypodium, and Silene, have been reported.

PEs have been reported from over 100 terrestrial plant families, representing ferns, gymnosperms, and angiosperms. The first isolation experiment of PE had a remarkable coincidence. Nakanishi et al. chemically investigated the leaves of Podocarpus nakaii (Podocarpaceae) and isolated three steroids, named ponasterones A (2), B (3) and C (4) (Fig. 1). Concurrently, an Australian group reported the isolation of 20-HE from the wood of Podocarpus elatus. These reports stimulated further research on different plant species for the exploration of other members of this class. A comprehensive list of PEs containing 212 new phytoecdysteroids with their plant sources is presented in Supporting Information Table S1.

3.1. Isolation and separation of phytoecdysteroids

The isolation and purification of major and minor ecdysteroids from plant material involve a multi-step procedure. This includes extraction, separation, and finally purification through different chromatography methods, including thin-layer chromatography, column chromatography, and high-performance column chromatography (HPLC). The polar nature of ecdysteroids makes it a complex process to separate them from other polar plant constituents, including polyphenolic compounds, chlorophyll, lipids, steroids, triterpenoids, pigment materials, and amino acids.

One of the common methods of the isolation of PEs is the solvent extraction of air-dried plant parts at room temperature, with a high excess of methanol or 95% ethanol. PEs were isolated from methanolic extract or 95% ethanol extract of the plant material followed by the removal of less polar organic compounds by partition with hexane and water. The residue of the aqueous portion was subjected to column chromatography through silica gel, alumina, Diaion HP-20, or Sephadex LH-20, and the fractions obtained from this chromatography were subjected to reverse phase HPLC using primarily silica gel 60G F254 or other silica gel columns as the stationary phase. Both aqueous methanol mixture and acetonitrile/trifluoroacetic acid mixture were used for the elution of the columns. In some cases, normal phase HPLC using dichloromethane/isopropyl alcohol/water mixture was effective for improved separation and isolation. Droplet countercurrent chromatography and rotation locular countercurrent chromatography techniques were also successful for separation. The details of chromatographic procedures for separation of PEs were previously reviewed.
3.2. Identification of phytoecdysteroids

The 14α-hydroxy-7-en-6-one chromophore of PEs showed characteristic ultra-violet absorption in methanol (MeOH) at $\lambda_{\text{max}}$ 240–245 nm. The proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy is limited in usefulness because it often gives spectra too complex to be analyzed. The $^{13}$C-NMR spectra with distortionless enhancement by polarization transfer experiments together with two-dimensional NMR (2D-NMR) experiments provide useful information regarding the skeletal structure. The key 2D-NMR includes correlation spectroscopy, heteronuclear single quantum coherence or correlation, heteronuclear multiple bond correlation, nuclear overhauser effect spectroscopy, and rotating frame nuclear overhauser effect spectroscopy. The carbon

Figure 2  Structures of reported new phytoecdysteroids (5–37) from Amaranthaceae family.
resonances of C-6 and C-20 appear to be the lowest at δC 201–204 and 207–209 ppm. The carbon resonances for C-2 and C-3 are near and δC 67–69 and C-14’s carbon resonances are near δC 83–85 ppm. Additionally, the carbon resonance values of C-7 and C-8 appear at δC 121–123 and 162–165 ppm. The 13C-NMR spectra of some PEs have already been reviewed. However, it is necessary to include that there is no method in analytical chemistry that would be appropriate for characterization of PEs does not exist.
3.3. Structural diversity and distribution of phytoecdysteroids in plant kingdom

In nature, PEs exist either in free-state or conjugated form, with sugars as glycosides or with organic acids as esters (such as acetate, benzoate, cinnamate, p-coumarate, and crotonate), sulphates, and isopropyldiene or methyl ether formation. Among the sugars, glucose, galactose, and xylose are common. Although variations in the steroid ring structure are not substantial, the significant variations are found in the number, position, and orientation of hydroxyl groups and conjugating moieties. In a few cases, an extra oxo group may be located at the C-2, C-12, C-17, C-20, or C-22 position along with requisite C-6 position. Most of these structural modifications are found in different plant families, possibly due to their different uses of metabolites. The highlights of structural modifications of new PEs due to natural sources of variations for those reported after 1998 are mentioned in Supporting Information Table S1.

3.4. Biosynthesis of phytoecdysteroids

Plants synthesize PEs mainly from mevalonic acid via cholesterol in the mevalonate pathway of the plant cell, using acetyl-CoA as a precursor\(^{13}\). The important intermediates of the mevalonate pathway are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Higher plants are capable of producing IPP and DMAPP from pyruvate and glyceraldehyde-3-phosphate also via the non-mevalonate pathway\(^{12}\). In the mevalonate pathway, the condensation reactions of six activated isoprene units form squalene and further undergo epoxidation and cyclization to form lanosterol. Additionally, the units undergo subsequent steps to form cholesterol\(^{15}\). Still, limited reports suggest the biosynthesis of PEs, yet with an unclear mechanism. The presence of PEs in various plant parts at different concentrations does not allow any conclusion to be drawn about the organ in which these compounds are primarily produced. Grebenok and Alder\(^{38}\) demonstrated that these compounds are possibly biosynthesized in developing tissues. The labelling experiments with \(^{14}\)C-mevalonic acid showed a prospective pathway involving the mevalonate pathway. Although the non-mevalonate pathway has not been reported, it is likely that 1-deoxy-o-xylulose could be used as an intermediate in mevalonate-independent deoxy-xylulose phosphate pathway (DOXP). Polypodium vulgare 20-HE might be biosynthesized from a \(\Delta 7\) sterol with a reduced side chain at C-24; this was shown in spinach, yet the involvement of sterols was not explored\(^{38}\). Feeding experiments with labelled cholesterol in Taxus baccata and P. vulgare produced labelled ecdysone and 20-HE\(^{39}\). The location of the radiolabel in biosynthesized 20-HE indicated that hydrogen migration occurred from the 3\(\alpha\)- and 4\(\beta\)-positions to C-4 and C-5 by concomitant 1,2-Wagner-Meerwein hydride migrations from the 4\(\beta\)-to the 5\(\beta\)- and from the 3\(\alpha\)-to the 4\(\alpha\)-position. The biosynthetic pathway study by Hyodo and Fujimoto\(^{37}\) in Ajaga reptans hairy roots indicated that 3\(\beta\)-hydroxy 5\(\beta\)-cholestan-6-one is converted into 2\(\beta\),3\(\beta\)-dihydroxy 5\(\beta\)-cholestan-6-one and the latter is converted to 20-HE, suggesting that 7-ene can be introduced at a later stage in the biosynthetic pathway. Based on the biosynthetic studies by several groups, it can be concluded that 20-HE can be biosynthesized from cholesterol as well as mevalonic acid in plants following the pathway as illustrated in Fig. 9.

4. New, naturally occurring phytoecdysteroids reported since 1999

This study comprehensively summarizes 212 new phytoecdysteroids reported from 17 plant families from 1999 to 2019, along with various bioactivities of PEs. The new phytoecdysteroids are numbered from 5 to 216 and the structures of those PEs are presented in Figs. 2–8. These 18 plant families include Amaranthaceae, Asteraceae, Blechnaceae, Caryophyllaceae, Clavicipitaceae, Commelinaceae, Dioscoreaceae, Gelsemiaceae, Lamiaceae, Liliaceae, Limnanthaceae, Lygodiaceae, Malvaceae, Menispermaceae, Polypodiaceae, Polypropaceae, Rhodomelaceae, and Taxaceae. Out of the reported 212 new PEs, 33 are from the Amaranthaceae family (5–37, Fig. 2), 50 are from the Asteraceae family (38–87, Fig. 3), five are from the Blechnaceae family (88–92, Fig. 4), 36 are from the Caryophyllaceae family (93–128, Fig. 5), one is from the Clavicipitaceae family (129, Fig. 6), 18 are from the Commelinaceae family (130–147, Fig. 6), one is from the Dioscoreaceae family (148, Fig. 6), three are from the Gelsemiaceae family (149–151, Fig. 6), 29 are from the Lamiaceae family (152–180, Fig. 7), one is from the Liliaceae family (181, Fig. 8), one is from the Limnanthaceae family (182, Fig. 8), one is from the Lygodiaceae family (183, Fig. 8), five are from the Malvaceae family (184–188, Fig. 8), seven are from the Menispermaceae family (189–195, Fig. 8), ten are from the Polypodiaceae family (196–205, Fig. 8), five are from the Polypropaceae family (206–210, Fig. 8), three are from the Rhodomelaceae family (211–213, Fig. 8), and three are from the Taxaceae family (214–216, Fig. 8).

**Figure 4** Structures of reported new phytoecdysteroids (88–92) from Blechnaceae family.
4.1. Amaranthaceae

The conducted literature survey indicates that 33 new ecdysteroids have been reported from the Amaranthaceae family. The new ecdysteroids are 2,22-dideoxy-20-hydroxyecdysone 25-\(\beta\)-D-gluco.pyranoside (5), 2,22-dideoxyecdysone 25-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)2)-\(\beta\)-D-glucopyranoside (6), 2,22-deoxyecdysone 25-\(\beta\)-D-glucopyranoside (7), (5\(\alpha\))-2,22-dideoxyecdysone 25-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)2)-\(\beta\)-D-glucopyranoside (8), 2,22-dideoxy-5\(\beta\)-hydroxyecdysone 25-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)2)-\(\beta\)-D-glucopyranoside (9), isolated from the plant Froelichia floridana, 20,26-dihydroxy 28-methyl ecdysone (10), 20,26-dihydroxy 24(28)-dehydro ecdysone (11), 20-hydroxyecdysone 22-glycolate (12), kancollosterone (13), isolated from the plant Chenopodium quinoa, 3\(\beta\),14\(\alpha\)-
dihydroxy-5β-pregn-7-one-2,6,20-trione (14), 24,25-dehydro-dronokosterone (15), 25,27-dehydroinokosterone (16), 5β-hydroxy-24(28)-dehydroinakisterone A (17), isolated from *Chenopodium album* Willd [44, 45], niuxixinsterone A [(20R,22R,24S)-20-O,22-O-(5'-hydroxymethyl)-furfurylidene-2β,3β,14α,25-tetrahydroxy-5β-ergost-7-en-6-one] (18), niuxixinsterone B [(20R,22R)-20-O,22-O-(5'-hydroxymethyl)-furfurylidene-2β,3β,25-trihydroxy-14β-methyl-18-nor-5β-cholesta-7,12-dien-6-one] (19), niuxixinsterone C [(20R,22R,25R)-20-O,22-O-(5'-hydroxymethyl)-furfurylidene-2β,3β,5β,14α,26-pentahydroxycholest-7-en-6-one] (20) [45], niuxixinsterone D (21) [45], (255)-20,22-O-(R-ethylidene) inokosterone (22), 20,22-O-(R-3-methoxycarbonyl) propylidene-20-hydroxyecdysone (23) [48], achyranthes A [(2β,3β,14α,20S,21,22R,25-heptahydroxycholest-7-en-6-one] (24) [48], (20R,22R)-2β,3β,20,22,26-pentahydroxy-cholestan-7,12-dien-6-one] (25) [48], isolated from the plant *Achyranthes bidentata* [49], aervecdysteroid A [20,25-epoxy-2β,3β,14α,22β-tetrahydroxy-5β-ecdysteroid] (26), aervecdysteroid B [24,28-dehydro-20,25-epoxy-2β,3β,14α,22β-tetrahydroxy-5β-ecdysteroid] (27), aervecdysteroid C [1β,3β,14α,20β,22β,25-hexahydroxy-5β-ecdysteroid] (28), aervecdysteroid D [24,28-dehydro-1β,3β,14α,20β,22β,25-hexahydroxy-5β-ecdysteroid] (29), isolated from the plant *Aerva javanica* [40], 2,3-isopropylidene cyasterone (30), 24-hydroxycyasterone (31), 2,3-isopropylidene isocyasterone (32), isolated from *Cyathula officinalis* Kuan [41], pfaffiaglycosides C (33),

Figure 6 Structures of reported new phytoecdysteroids (129–151) from Clavicipitacae, Commelinaceae, Dioscoreaceae, and Gleicheniaceae families.
pfaffiaglycosides D (34), pfaffiaglycosides E (35), isolated from *Pfaffia glomerata*\(^5\), (20R)-22-deoxy-20,21-dihydroxyecdysone (36), isolated from *Rhagodia baccata* (Labill.) Moq.\(^5\), and septaneocydysone (37), isolated from the plant *Atriplex portulacoides* L.\(^5\).

### 4.2. Asteraceae

The literature survey indicates that 50 new ecdysteroids have been reported from the Asteraceae family. These are inokosterone 20,22-acetonide (38), integristerone A-20,22-acetonide

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**Figure 7** Structures of reported new phytoecdysteroids (152–180) from Lamiaceae family.
(39), 15-hydroxyponasterone A (40), 14-epi-ponasterone A 22-O-β-D-glucopyranoside (41), carthamoleusterone (42), 22-deoxy-28-hydroxymakisterone C (43), 26-hydroxymakisterone C (44) and 1β-hydroxymakisterone C (45), isolated from the plant *Leuzea carthamoides*56, lesterone [5β-cholest-7-en-2α,3α,11β,14α,20R,22R,25-heptahydroxy-6-one] (46), isolated from the plant *L. carthamoides*57, leuzeasterone (47) and (24Z)-29-hydroxy-24(28)-dehydromakisterone C (48) isolated
from the plant *L. carthamoides* [58], coronatosterone (2-deoxy-3-epi-4β,20-dihydroxyecdysone) [(20R,22R)-3α,4β,14a,20,22,25-hexahydroxy-5β-cholestan-7-en-6-one] [49], isolated from *Serratula coronate* [59], 20-hydroxyecdysone-2-O-β-D-galactopyranoside (50), 3-O-acetyl-20-hydroxyecdysone-2-O-β-D-galactopyranoside (51), 3-O-acetyl-20-hydroxyecdysone-2-O-β-D-glucopyranoside (52), 24-O-acetyl-epi-abutasterone (53), 20-hydroxyecdysone-20,22-butylidene acetal (54), isolated from the plant *Serratula chinensis* [60, 61], rhapontisterone R 1 [2β,3β,11α,14α,20R,22R-hexahydroxy-stigma-7,24(28)-dien-6-oxo-28,25-carbolactone] (55), isolated from *Rhaponticum uniflorum* [62], turkesterone-2-O-cinnamate (56), isolated from *R. uniflorum* [63], makisterone C-20,22-acetonide (57), isolated from *R. uniflorum* [64], ajugasterone C-2,3,20,22-diacetone (58) and 5-deoxykaladasterone-20,22-monoacetone (59), isolated from *R. uniflorum* [65], uniflorostereone (60), isolated from *R. uniflorum* [66], rapisterone D 20-acetate (61), isolated from *L. carthamoides* [67], 22-epi-ajugasterone C (62), isolated from *Serratula cichoracea* [58], ajugasterone 11-acetate (63) [69], 3-epi-20-hydroxyecdysone (64) [70], ecdysone 22-acetate (65), (25S)-inokosterone 26-acetate (66), 20,22-O-(R-ethylidene)-20-hydroxyecdysone (67) and 20,22-O-(R-ethylidene)-ajugasterone C (68), isolated from *S. coronate* L. [71], 25,26-didehydroponasterone A (69) and stachyasterone C (70), isolated from *Klaseopsis chinensis* [72], 11α-hydroxypoststerolone (71) [73], herkest erone [53,25-dihydroxyacryhanosterone] (72) [74], 25-hydroxyacryhanosterone (73) [75], 14-epi-20-hydroxyecdysone (74) [76], 2β,3β,20R,22R,25-pentahydroxy-5β-cholestan-6,8(14)-dien (75) [77], 24-methylene-shidasterone (76) [78], 14α,15α-epoxy-14,15-dihydrostachyasterone B (77) [79], 20,22-didehydrotaxisterone (78) [80], 1-hydroxy-20,22-didehydrotaxisterone (79) [81], serfurosterone A (80) [82], serfurosterolone B (81) [83], 14α,15α-epoxy-20R,22R,2β,3β,20,22,25-pentahydroxy-5β-cholesta-7,14-dien-6-one (82) [84], (20R,22R)-2β,3β,20,22,25-pentahydroxy-5β-cholesta-7-en-6-one (83) [85], 22-methylene-2β,3β,11α,14α,25-pentahydroxy-5β-cholesta-7-en-6-one (84) [86], 2β,3β,14α,25-tetrahydroxy-5β-cholesta-7,20(22)-dien-6-one (85) [87] and 1β,2β,3β,14α,25-pentahydroxy-
5β-chol-7,20(22)-dien-6-one (86), isolated from Serratula wolffii, and (24R)-24-(2-hydroxyethyl)-20-hydroxycydysone (87) isolated from the plant Serratula stragulata.

4.3. Blechnaceae

The Literature survey indicates that five new ecdysteroids have been reported from the Blechnaceae family. These are braineosteride A [14-deoxy-14α-15α-epoxyponasterone A] (88), brainesteride B [14-deoxy-14α,15,16-didehydroponasterone A] (89), brainesteride C [25-deoxyecolstone-3-β-D-glucopyranoside] (90), brainesteride D [25-deoxyecdysone-3-β-D-glucopyranoside] (91), and brainesteride E [5-epi-ponasterone A] (92) isolated from the plant Brainea insignis.

4.4. Caryophyllaceae

Thirty-six new ecdysteroids have been reported from the Caryophyllaceae family. The new ecdysteroids are integrineste A 25-acetate (93), isolated from Silene brahuiaca (94), japonicone (22,25-epoxy-24-methylene-3,14,20-tetrahydroxycholest-7-en-6-one) (94), isolated from Sagina japonica (95), 2-dehydroxyecdysone-3-β-2-benzoxoate (95) (96), 2-deoxyecdysterone-25-acetate (96) (97), isolated from Silene wallichiana, 5α-2-deoxy-20-hydroxyecdysone 20,22-acetonide (97) (98), isolated from Silene viridiflora, (11α)-11-hydroxyecdysisone ([2β,3β,5β,11α,22R]-22,25-epoxy-2,3,11,14,20-pentahydroxycholest-7-en-6-one) (99), (2β,3β,5β,14α,22R)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (100), (2β,3α,5β,14α,22R)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (101), 2-dehydro-20-deoxy ajugasterone C (102), 1-hydroxy-2-deoxy-20,21-didehydro ecdysone (103), 2-deoxy-20,21-didehydro ecdysone (104), ponasterone A-22-apiside (2β,3β,14α,20R-tetrahydroxy-[3,4- (hydroxymethyl)]-tetryadrofuran-2-yl]oxo)-cholest-7-en-6-one (105), 3-epi-shidasterone [22,25-epoxy-2β,3α,14α,20R-tetrahydroxy-5β-cholest-7-en-6-one (106), isolated from S. wolffi (106), 26-hydroxyintegristerone A (107), isolated from Silene frivaldskyana, 2-deoxy-20-hydroxyecdysone 25-glucoside (108), isolated from Silene gigantea, 20,26-dihydroxyecdysone (109), 5α,20,26-trihydroxyecdysone 20,22-acetonide (110), 20,26-dihydroxyecdysone 22,22-acetonide (111), 20,26-dihydroxyecdysone 22,22-acetonide (112), 20-hydroxyecdysone 20,22-monoacetone-25-acetate (113), 22,22-diacetate-20,26-dihydroxyecdysone (114), 3,22-diacetate-20,26-dihydroxyecdysone (115), isolated from S. viridiflora (90-92), 22-O-α-D-galactosylintegisterone A 25-acetate (sileneoside H) (116), isolated from S. brahuiaca, 9α,20-dihydroxyecdysone (117), 9β,20-dihydroxyecdysone (118), isolated from Silene italic ssp. nemoralis, 3-β-α-D-glucopyranosyl-β-D-glucopyranosyl-3-β-α-D-glucopyranosyl-β-D-glucopyranosyl-3-β-α-D-glucopyranosyl-7-β-D-glucopyranosyl-cholest-7-en-6-one, 25-O-α-D-glucopyranosyl (119), isolated from Silene montbretiana, 23,23-diacetate-22-benzoxo-20-hydroxyecdysone (120), isolated from Silene guttens dens B. Fedtsch, 2-deoxyecdysterone 22β-β-glucoside (121), 2-deoxy-20,26-dihydroxyecdysone (122) and 2-deoxyypolypodine B 3β-α-glucoside (123), isolated from Silene pseudotites, 2,2-deoxy-21-hydroxyecdysone (124) and 5α-2-deoxy-21-hydroxyecdysone (125), isolated from Silene ottica, 3α,14α,22R,25-tetrahydroxy-5β(H)-cholest-7-en-6-one (126), isolated from Acanthophyllum gypsophilotes, and 22,22-dideoxy-20-hydroxyecdysone 3β-β-D-glucopyranoside (127), isolated from Cuculus baccifer and 2-deoxy-20-hydroxyecdysone-22-O-β-D-glucopyranoside (128), isolated from the plant Silene italic ssp. nemoralis.

4.5. Clavicipitaceae

One new ecdysteroid has been reported from the Clavicipitaceae family. The new ecdysteroid 22-dehydroxyecdysone (129) is isolated from Nomuraea rileyi (fungus).

4.6. Commelinaceae

Eighteen ecdysteroids have been isolated from the Commelinaceae family. These ecdysteroids are 11α-hydroxyrubrostereone (130), dacyrhanasterone (131), calonymonterone (132), 133, 200-ajugasterone B (134), 22-oxo-ajugasterone C (135), 22-oxo-20-hydroxyecdysone (136), 22-oxo-20-hydroxyecdysone (137), shidasterone-3-acetate (138), isolated from the plant Cyanotis achmeoides, 3β,4α,14α,20R,22R,23,25,26-hydroxycholest-7-en-6-one (139), isolated from the plant C. achmeoida B. Clarke, 11β,20-oxo-20-hydroxyestrone (140), 14,15-dehydro-22-oxo-2-acetate (141), 22-oxo-2-acetate (142), 24-epi-24-oxo-2-acetate (143), 24-epi-ajugasterone C (144), isolated from the plant Cyanotis longifolia, calledcdysterol B (145), calcedysterol B (146), and callcedysterol C (147), isolated from the plant Callisia fragrans.

4.7. Dioscoreaceae

The conducted literature survey indicates that one new ecdysteroid, namely (20R)-5β-11α,20-trihydroxyecdysone (148), has been isolated from the plant Dioscorea dumetorum (Dioscoreaceae).

4.8. Gleicheniaceae

The literature survey indicates that three new ecdysteroids, (22R,24R,25S,26S)-2β,3β,14α,20R-tetrahydroxy-26-oxo-6-4-oxo-7-stigmaster-7-e-22,26-lactone (149), (22R,24R,25S)-2β,3β,14α,20R,22R,23,25,26-pentahydroxy-6-oxo-7-stigmaster-7-e-22,26-lactone (150), and (22R,25S)-2β,3β,14α,20R,24S-pentahydroxy-6,26-dioxo-stigmaster-7-e-22,26-lactone (151), have been isolated from the plant Diplopterygium rufofolium (Gleicheniaceae).

4.9. Lamiaceae

A literature survey indicates that 9 new ecdysteroids have been reported from the Lamiaceae family. These are 28-epi-cyasterone ((22R,24S,25S,26S)-5β-stigmaster-7-e-26-oic acid, 2β,3β,14α,20,22,26-26-hydroxy-6-oxo-γ-lactone) (152), isolated from Ericahyton wallACHI (153), ajugalide-E (153) from Ajuga taimensis, breviflorasterone (154), ajugacetalsterone C (155), ajugacetalsterone D (156) from Ajuga macroserpa var. breviflora, decumebesterone A (157) and ajugacetalsterone E (158), isolated from Ajuga decumens, 22-dehydrocysterone-2-glucoside (159), ajugacetalsterone A (160) and ajugacetalsterone B (161), isolated from Ajuga nipponensis, 25-hydroxy-acystasterone A (162), 11-hydroxy-cyasterone (163), 11-hydroxy-sterone (164), turkesterone 22-acetate (165), 22 oxo-turkesterone (166), 11-hydroxy-Δ24-capitasterone (167) and turkesterone 20,22-acetonide (168), isolated from Ajuga turkestiana, 11β,20-oxo-21-hydroxydihydasterone (172), 11β,20-oxo-20-deoxysterone (173) and 2,3-acetonide-24-hydroxyecdysone (174), isolated from Vitex doniana, 24-epi-pinnatasterone (175) and scabrambesterone (176), isolated from Vitex scabra, 26-
hydroxypinnasterone (177), isolated from Vitex cynosa125, (24R)-11α,20,24-trihydroxyecdysone (178)126, and 11α,20,26-trihydroxyecdysone (179)127 and 24-methylhidastereone (180)127, isolated from Vitex canescens.

4.10. Liliaceae

The literature survey indicates that one new ecdysteroid, stachyosterone A-20,22-acetonide (181), has been isolated from Asparagus fuscatus Buch.-Ham. (Liliaceae)128.

4.11. Limnanthaceae

The literature survey indicates that one new ecdysteroid, namely limnanthes alba limnantheoside C (20-hydroxyecdysone 3-O-β-D-glucopyranosyl- [1→3]-β-D-xylopyranoside) (182), has been isolated from Limnanthes alba Hartw. (Limnanthaceae)129.

4.12. Lygodiaceae

The literature survey indicates that one new ecdysteroid, lygiodiumsteroid A (183), has been isolated from Lygodium japonicum (Thunb.) Sw. (Lygodiaceae)130.

4.13. Malvaceae

Five new ecdysteroids have been reported from the Malvaceae family. These new ecdysteroids are 25-acetoxy-20-hydroxyecdysone-3-O-β-D-glucopyranoside [(2β,3β,5β,22R)-3-[(β-D-glucopyranosyl)oxy]-2,14,20,22-tetrahydroxy-6-oxo-cholest-7-en-25-yl acetate] (184), sterosterone-3-O-β-D-glucopyranoside [(2β,3β,5β,22R,24S)-2,14,20,22,24-penta hydroxy-6-oxo-chole st-7-en-3-yl β-D-glucopyranoside] (185), ecdysone-3-O-β-D-glucopyranoside [(2β,3β,5β,22R)-2,14,22-trihydroxy-6-oxo-cholest-7-en-3-yl β-D-glucopyranoside] (186), isolated from the plant Sida rhombifolia131, 20-hydroxy-24-hydroxymethyl ecdysone (187), isolated from the plant Sida spinosa132, and glutinosterone [(20R,22R)-1β,2β,3β,14α,20,22,25-heptahydroxy-5β-cholest-7,24-dien-6-one] (188), isolated from the plant Sida glutinosa133.

4.14. Menispermaceae

The literature survey indicates that seven new ecdysteroids have been reported from the Menispermaceae family. These are sphenocentroside A (189) and sphenocentroside B (190), isolated from Sphenocentrum jollyanum34, cycleasterone A (191), isolated from Cylindranthus xilifolius35, 3-deoxy-20,24-dihydroxyecdysone (192), 2-deoxy-5β,20-dihydroxyecdysone (193), and diplocidone (194) from Diploclisia glaucescens136,138 and fibraeecdyside A (195), isolated from Fibraeecdyside tinctoria Lour139.

4.15. Polypodiaceae

The literature survey indicates that ten new ecdysteroids have been reported from Polypodiaceae family. The new ecdysteroids are 5-hydroxyecdysone (196), 20-deoxyshidasterone (197) and polydine B 2-β-D-glucoside (198), isolated from the plant P. vulgaris140, 20-deoxymakisterone A (199), 25-epimer of amarasterone A (200), 25-deoxymakisterone-22-β-D-glucoside (201) from Microsorum scolopendria141, E-2-deoxy-20-hydroxyecdysone-3-[4-(1-β-D-glucopyranosyl)]-caffeate (202), 2-deoxyecdysone-3-[4-(1-β-D-glucopyranosyl)]-ferulate (203), 2-deoxyecdysone 25-α,1-ramhomypanoside (204), isolated from Microsorum membranifolium142,143 and polanosteride B (205), isolated from Lepidogrammitis drymoglossoides144.

4.16. Polyporaceae

From the Polyporaceae family, the conducted literature survey indicates that five new ecdysteroids, (20S,20R,24R)-16,22-epoxy-3,14α,23β,25-tetrahydroxyergost-7-en-6-one (206), (23R, 24R,25R)-23,26-epoxy-3β,14α,20α,22α-tetrahydroxyergost-7-en-6-one (207), polyporoid A (208), polyporoid B (209), and polyporoid C (210) are all isolated from the plant Polyporus umbellatus185,186.

4.17. Rhodomelaceae

A literature survey indicates that three new ecdysteroids have been reported from the Rhodomelaceae family. These are alfredensterol (211), 3-deacetoxy alfredensterol (212), and 14α-hydroxy alfredensterol (213), isolated from Laurencia alfredensis (red algae)147.

4.18. Taxaceae

From the Taxaceae family, a conducted literature survey indicates that three new ecdysteroids have been reported. The new ecdysteroids are 7,8β-dihydroponasterone A (214), isolated from the plant Taxus cuspidate148, polanosterone A 20,22-p-hydroxybenzylidene acetal (215), and polanosterone A 20,22-acetonide (216), isolated from the plant Taxus canadensis Marsh149.

5. Biological and pharmacological activities of phytoecdysteroids

PEs play prominent roles in growth regulation and in the protection of plant species; they may have potential therapeutic applications. Various pharmacological effects of new PEs based on in vitro and in vivo studies are presented in Table 1. PEs have been known for their numerous bioactivities relating to their broad-spectrum general health-promoting effects. They are known for their numerous beneficial effects on mammals, which include as anabolic, adaptogenic, anti-diabetic, hypolipidemic, and hepatoprotective effects150-153. The prospective results demonstrate that PEs can be considered efficacious against several acute and chronic pathophysiological conditions as discussed further.

5.1. Antioxidant activities

The antioxidant properties of PEs have been reported to display beneficial effects in various chronic conditions. A novel phytoecdysterone ajugaceratolone E (158) exhibited in vitro cytotoxic activity, superoxide anion generation, and elastase release in N-formyl-methionyl-leucyl-phenylalanine/cytocatalasin B (FMLP/CB)-induced human neutrophils154,211, 20,26-dihydroxy-28-methyl ecdysone (10), 20,26-dihydroxy-24(28)-dehydro ecdysone (11), and 20-hydroxyecdysone 22-glycolate (12), isolated from C. quinoa seeds, have exhibited inhibitory effects on calf skin collagenase and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Hence, these ecdysteroids may be considered as natural chemicals to prevent and delay both collagenase-related skin damages and oxidative stress155-157. Another PE derivative cyasterone along with chikusetsusaponin IV could significantly inhibit inflammatory and oxidative enzyme levels, such as thromboxane A2 (TXA2), endothelin (ET), malondialdehyde
| Name of the ecdysteroids | Pharmacological activity | Study model | Cell line used | Conc./dose | Effect | Mechanism |
|--------------------------|--------------------------|-------------|----------------|------------|--------|-----------|
| 2,22-Dideoxy-20-hydroxyecdysone 25-O-β-D-glucopyranoside (5), 2,22-dideoxyecdysone 25-O-β-D-glucopyranosyl-(1→2)-β-α-glucopyranoside (6) 20,26-Dihydroxy 28-methyl ecdysone (10), 20,26-dihydroxy 24(28)-dehydro ecdysone (11), 20-hydroxyecdysone 22-glycolate (12) | Evaluated human Topo I inhibition and no significant effect have been observed. | In vitro | Escherichia coli | 30–312 μmol/L | Growth inhibition | Topoisomerase I inhibition |
| 20,26-Dihydroxy 28-methyl ecdysone (10), 20,26-dihydroxy 24(28)-dehydro ecdysone (11), 20-hydroxyecdysone 22-glycolate (12) 3β,14α,5β,22,26,20-triene-2,6,20-trione (14) | Evaluated inhibitory effect on calf skin collagenase and scavenging DPPH free radicals, as well as in chelating the iron metal ions and significant effect have been observed. | In vitro | Clostridium histolyticum | DPPH assay: 5–30 μg/mL; metal ion chelation: 10–70 μg/mL; collagenase assay: 5–100 μg/mL | Antioxidant | Free-radical-scavenging; collagenase inhibition |
| Niuxixinsterone D (21) | Evaluated inhibitory effects against LPS-induced NO production in RAW 264.7 macrophages and exhibited anti-neuroinflammatory activity with inhibited 29.7 and 26.0% NO production. | In vitro | RAW 264.7 macrophages | 50 μmol/L | Anti-inflammatory | Inhibition of nitric oxide production |
| Aervedcysteoid A (20,25-epoxy-2β,3β,14α,22β-tetrahydroxy-5β-ecdysteroid) (26), aervedcysteoid B (24,28-dehydro-20,25-epoxy-2β,3β,14α,22β-tetrahydroxy-5β-ecdysteroid) (27), aervedcysteoid C (1β,3β,14α,20β,22β,25-hexahydroxy-5β-ecdysteroid) (28), aervedcysteoid D (24,28-dehydro-1β,3β,14α,20β,22β,25-hexahydroxy-5β-ecdysteroid) (29) | Evaluated enzyme inhibitory activities against AChE, BChE and LOX and no significant effect have been observed. | In vitro | Ovine seminal vesicles microsomes | 100 μmol/L | Anti-inflammatory | Inhibition of AChE, BChE and LOX enzymes |
| Septanoecdysone (37) | Evaluated agonistic activity in the Drosophila melanogaster BII cell bioassay and shown good activity. | In vitro | Drosophila melanogaster BII cell bioassay | ED_{50} 20 μmol/L | Agonistic activity | Growth inhibition |
| Septanoecdysone (37) | Evaluated antioxidant activity by using DPPH, ABTS⁺, Fe³⁺ and catalase assays. Also evaluated antibacterial and anticholinesterase activities and shown significant activities. | In vitro | E. coli; Human plasma | DPPH: 0.062–1.0 mg/mL; ABTS: 2.45 mmol/L; others: 0.062–1.0 mg/mL | Antioxidant, antibacterial, anti-inflammatory | Free-radical-scavenging; ChE inhibition; growth inhibitory |

(continued on next page)
| Name of the ecdysteroids | Pharmacological activity | Study model | Cell line used | Conc./dose | Effect | Mechanism | Ref. |
|--------------------------|--------------------------|-------------|----------------|------------|--------|-----------|------|
| 22-epi-Ajugasterone C (62) | Weak DPPH radical-scavenging and antimicrobial activity against multi-resistant strains Gram positive bacteria *(Staphylococcus aureus)* 118, Gram negative bacteria such as *(Escherichia coli)* 113, *Klebsiella pneumoniae* 112, *Proteus mirabilis* 23, *Serratia sp.* 2028, *Pseudomonas aurogenosa* 43 and yeasts *(Candida albicans)*. | *In vitro* | *E. coli* | 1–300 µg/mL | Antioxidant, antimicrobial | Free-radical-scavenging; growth inhibitory | 68 |
| 3-epi-20-Hydroxyecdysone (64), ecdysone 22-acetate (65), (25S)-inokosterone 26-acetate (66), 20,22-O-(R-ethylidene)-20-hydroxyecdysone (67), 20,22-O-(R-ethylidene)-ajugasterone C (68) | Evaluated biological activity in the agonist version of the *Drosophila melanogaster* BII cell bioassay. | *In vitro* | *Drosophila melanogaster* BII cell line | EC_{50} 16, 1.1, 0.6, 43, and 62 µmol/L | Agonistic activity | Growth inhibition | 70,71 |
| 20,22-Didehydro taxisterone (78), 1-hydroxy-20,22-didehydrotaxisterone (79), (24R)-24(2-Hydroxyethyl)-20-hydroxyecdysone (87) | Evaluated toxicity in the oral aphid *Acyrthosiphon pisonium* (Harris) test and exhibited low toxicity. | *In vitro* | *(Acyrthosiphon pisonium)* Harris | LC_{50}> 100 ppm; LC_{50} = 48.5 ppm | Oral aphid test | Cytotoxic activity | 76 |
| 3-O-β-D-Glucopyranosyl-3β,25-dihydroxy-5β-cholest-7-en-6-one-25-O-β-D-glucopyranoside (119) | Evaluated cytotoxicity against two cancer cell lines of A549 (human lung adenocarcinoma) and HeLa cells (human epithelial cervix carcinoma) by using the MTT assay and no significant activity have been observed. | *In vitro* | Lung cancer cell line (A549); leukemia cell line (HeLa) | 12.5–100 µmol/L | Cytotoxic activity | Growth inhibitory | 96 |
| 2,3-Diacetate-22-benzote-20-hydroxyecdysone (120) | Evaluated cytotoxicity, antiproliferative activities in HeLa, HepG-2, and MCF-7 cell lines and antioxidant activities and showed moderate activities. | *In vitro* | Human cervix adenocarcinoma (HeLa), hepatocellular carcinoma (HepG-2), breast adenocarcinoma (MCF-7) cells | 1–500 µg/mL | Cytotoxic activity; antiproliferative; antioxidant | Cell growth inhibition; free-radical-scavenging | 97 |
| 3α,14α,22R,25-Tetrahydroxy-5β(H)-cholest-7-en-6-one (126) | Evaluated anti-inflammatory and analgesic activities. | *In vivo* | Acetic acid induced inflammation in rat | 50 mg/kg | Anti-inflammatory, analgesic activities | Inhibition of peritoneal exudation and irritation | 100 |
| **Phytoecdysteroids in health and disease** |
|---------------------------------------------------------------|
| 
| **(20R)-5β-11α,20-Trihydroxyecdysone (148)** | Evaluated antifungal activity against three Candida species viz. *Candida albicans*, *Candida glabrata* and *Candida tropicalis* and no significant effect have been observed. |  | In vitro | *Candida albicans*, *Candida glabrata* and *Candida tropicalis* | MIC: >200 μg/mL | Antifungal activity | Growth inhibition 113 |
| **(22R,24R,25S,26S)-2β,3β,14α,20R-Tetrahydroxy-26α-methoxy-6-oxo-stigmaster-7-ene-22,26-lactone (149)**, **(22R,24R,25S)-2β,3β,14α,20R,26S-Pentahydroxy-6-oxo-stigmaster-7-ene-22,26-lactone (150)** | Evaluated antifungal and antibacterial properties by disk diffusion method and exhibited moderate antibacterial activity against oral pathogens. |  | In vitro | *Candida albicans*, *C. tropicalis*, *C. glabrata*, *Streptococcus mutans*, and *S. viridans* cultures | MIC: 48–72 mg/mL | Antifungal and antibacterial activities | Growth inhibitory 114 |
| **Decumbesterone A (157)** | Evaluated inhibitory effects on EBV-EA induction by the tumorpromoter (TPA) as a primary screening test for antitumor-promoters and showed very strong inhibitory effects. |  | In vivo | DMBA/TPA-induced skin tumor in mice | 1–32 nmol | Inhibitory effects | Inhibition of Epstein-Barr virus early antigen 118 |
| **Ajugacetalsterone E (158)** | Evaluated cytotoxic activity against MCF-7, HepG2, A549, and B16 cell lines using MTT method and the inhibition of superoxide anion generation and elastase release and moderate activity have been observed. |  | In vitro | MCF-7, HepG2, A549, B16 cell lines | 10–48 μmol/L | Cytotoxic and antioxidant activity | Growth inhibition, inhibition of SOD generation and elastase release 119 |
| **Ajugacetalsterone A (160)** | Evaluated cytotoxicity against human lung cancer cell A-549, human hepatoma cell HepG2, human breast cancer cell MCF-7 and proliferating epidermal carcinoma cell HeLa using MTT method and significant activity have been observed. |  | In vitro | A-549, HepG2, MCF-7 cell lines | IC_{50} 26.5 ± 1.3, 33.3 ± 1.4, 25.4 ± 1.8 and 28.1 ± 2.9 μmol/L | Cytotoxic activity; antiproliferative | Cell growth inhibition 170 |
| **24-epi-Pinnatasterone (175), scabrasterone (176)** | Evaluated Musca assay for moulting hormone activity and exhibited very low activity. |  | In vivo | Musca bioassay | EC_{50}: 0.5, 1 μmol/L | Susceptibility activity | Moulting hormone activity (continued on next page) 124 |
| Name of the ecdysteroids | Pharmacological activity | Study model | Cell line used | Conc./dose | Effect | Mechanism | Ref. |
|--------------------------|--------------------------|-------------|----------------|------------|--------|-----------|------|
| Stachysterone A-20,22-acetonide (181) | Evaluated cytotoxicity against human breast adenocarcinoma MDA-MB-231 cell line and no significant activity have been observed. | In vitro | Human breast adenocarcinoma MDA-MB-231 cell line | 2–50 μmol/L | Cytotoxicity activity | Growth inhibitory, inhibition of cell proliferation | 128 |
| Glutinosterone (188) | Evaluated liver function in terms of serum enzyme activity, lipid metabolic enzyme and antibacterial activity and exhibited significant activity. | In vitro | Human blood cells | 5–25 μg/mL | Liver function improvement, antibacterial activity | Inhibition of hepatotoxicity markers SGOT, SGPT and ALP | 133 |
| Sphenocentroside A (189), sphenocentroside B (190) | Screened antimicrobial activity and moderate activity have been observed. | In vitro | E. coli | 20 μg/mL | Antimicrobial activity | Growth inhibition | 134 |
| Cycleasterone A (191) | Evaluated hepatoprotective activity against APAP-induced toxicity in HepG2 cells and moderate activity have been observed. | In vitro | N-acetyl-p-aminophenol (APAP)-induced toxicity in HepG2 cells | 10 μmol/L | Hepatoprotective activity | Improving cell survival | 135 |
| Fibraurecdyside A (195) | Evaluated inhibitory effects against cytochrome P450 3A4 (CYP3A4) and no significant activity have been observed. | In vitro | E. coli | 1–2 μmol/L | Growth inhibitory, antioxidant activity | Inhibition of cytochrome P450 3A4 (CYP3A4) | 139 |
| Polyporoid A (208), polyporoid B (209), polyporoid C (210) | Evaluated anti-inflammatory activity against TPA-induced inflammation in mice and exhibited significant inhibitory activity. | In vivo | TPA-induced inflammation in mice | ID50 0.1–1 μmol/L | Anti-inflammatory activity | Inhibition of topical skin inflammation | 146 |
| Alfredensterol (211), 3-deacetoxy alfredensterol (212), 14α-hydroxy alfredensterol (213) | Evaluated antiproliferative activity against MDA-MB-231 breast and HeLa human cervical cancer cell lines and exhibited moderate activity. | In vivo | MDA-MB-231, HeLa cell lines | MDA-MB-231, IC50 = 27.6, 21.6 and 15.8 μmol/L; HeLa IC50 = 25.6, 43.5 and 48.2 μmol/L | Anti-proliferative activity | Inhibition of cell growth and proliferation | 147 |

AAPH, 2,2’-azobis(2-amidinopropane) dihydrochloride; ABTS+, 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate); AChE, acetylcholinesterase; APAP, N-acetyl-p-aminophenol; BChE, butyrylcholinesterase; CYP3A4, cytochrome P450 3A4; EBV-EA, Epstein-Barr virus early antigen; EC50, half maximal effective concentration; ID50, median infectious dose; LC50, lethal concentration 50; LOX, lipoxygenase; MIC, minimum inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RBC, red blood cell; TPA, 12-O-tetradecanoylphorbol-13-acetate.
(MDA), and cyclooxygenase-2 (COX-2) as well as promote the activities of endothelial nitric oxide synthase (eNOS) and superoxide dismutase (SOD)\textsuperscript{144}. PE 22-epi-ajugasterone C (62) also showed in vitro antioxidant activity by DPPH radical scavenging assay\textsuperscript{68}.

5.2. Anti-inflammatory effects

Antioxidant and antimicrobial effects are often associated with anti-inflammatory responses, and such a combination of bioactivities was also reported for several pytoecdysteroids. The methanolic extract of stem bark of \textit{V. domiana} led to purification of three new PEs: (21-hydroxyshidasterone, 11\textbeta}-hydroxy-20-deoxyshidasterone, and 2,3-acetone-24-hydroxyecdysone) as well as several known PEs (ecdysteroids shidasterone, ajugasterone C, 24-hydroxyecdysone and 11\textbeta,24-hydroxyecdysone). The PEs 21-hydroxyshidasterone (172), 11\textbeta}-hydroxy-20-deoxyshidasterone (173), and 2,3-acetone-24-hydroxyecdysone (174) displayed significant inflammation-inhibitory effects on rat paw edema development induced by carrageenan in Sprague–Dawley rats\textsuperscript{125}. PE niuxixinsterone D (21) was tested for inhibitory effects against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophages. Niuxixinsterone D and serfruitosterone A showed anti-neuroinflammatory activity by inhibiting NO production by 29.7% and 26.0%, respectively\textsuperscript{47}. Another PE 3a,14a,22R,25-tetrahydroxy-5\textbeta(H)-cholesterol-7-en-6-one (126), isolated from \textit{A. gypsophioloides}, was shown to possess anti-inflammatory and analgesic activities\textsuperscript{100}. A recent report also showed various biological activities of PEs which were isolated from \textit{Silene genus}. \textalpha-Ecdysone, in particular, was investigated for possible immunomodulatory and anti-inflammatory effects on LPS-treated RAW 264.7 macrophages and in a zebrafish model\textsuperscript{55}. The compound showed potent immunostimulatory effects by enhancing membrane fluidity and lysosomal enzyme activity. \textalpha-Ecdysone simultaneously inhibited the levels of NO and suppressed the levels of pro-inflammatory mediators and cytokines. An intriguing mechanism of the action involved increased heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf-2) production and mitigation of nuclear factor-kappa-light-chain-enhancer of activated B (NF-\kappaB) activity, as well as a reduction in mitogen-activated protein kinases (MAPKs) and protein kinase B (Akt) activation\textsuperscript{55}. Some of the known PEs, such as paristerone, ecdysterone, and capitasterone isolated from the stems of \textit{Diplodisia glaucescens}, have shown significant anti-inflammatory activity with half maximal inhibitory concentration (IC\textsubscript{50}) values ranging from 1.5 to 11.6 \textmu M/L, respectively; this was discovered by measuring the inhibitory ratios of \beta-glucuronidase release in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factors\textsuperscript{55}. These observations overall conclude that diverse PE constituents exhibit notable anti-inflammatory effects (Fig. 10).

5.3. Tissue differentiation activities

Metabolic pathways related to anabolic activity associated with the biosynthetic processes forming molecules from smaller units. This mainly relates to the energy requirement as an endergonic process, and cell and tissue repair and regeneration. PEs have shown anabolic activity in several in vitro and in vivo studies with a characteristic increase in growth and skeletal muscle mass as well as an increase in fiber size through muscle-specific effects in different animals\textsuperscript{157}. 20-HE has been shown to improve dermal thickness\textsuperscript{151,158} and promote wound-healing \textit{in vivo}\textsuperscript{13,150}. Additionally, 20-HE was also found to enhance keratinocyte differentiation\textsuperscript{153}, increase protein synthesis \textit{via} Akt activation\textsuperscript{159}, and inhibit collagenase activity \textit{in vitro}\textsuperscript{152}. Furthermore, 20-HE has also been shown to reduce production of reactive oxygen species (ROS) induced by cobalt chloride and hydrogen peroxide in PC12 rat pheochromocytoma cells and B35 rat neuroblastoma cells, respectively\textsuperscript{160}. Complex phytochemical mixtures can exert diverse biochemical effects on cellular signaling pathways, protein synthesis, and enzymatic activities as presented in Fig. 11. The positive interactions between molecules, termed potentiation, can lead to additive or synergistic effects that enhance the individual bioactivity of a particular molecule\textsuperscript{161}. PEs also possess a promising future in skin care due to their perception as being a safe material, their abundance, and their sustainability\textsuperscript{162}. The three main significant biochemical targets in skin care include matrix metalloproteinases, the endopeptidases responsible for collagen breakdown, and other targets, including tyrosinase. Amongst these targets, tyrosinase is the key enzyme involved in skin pigmentation and ROS-mediated oxidative stress, exacerbating the aging process. Additionally, it has been discovered that PEs present in cosmetic preparation protect skin from pigmentation and aging\textsuperscript{163}. The probable mechanisms of such dermal effects need elucidation.

5.4. Metabolism-modulatory effects

PEs exert biomedical effects in different clinico-pathophysiological conditions through a variety of mechanisms. Recently, PE derivatives have shown their non-hormonal anabolic and adaptogenic effects in mammals, including humans\textsuperscript{17}, through a probable mechanism involving the activation of protein kinase B (also known as Akt)\textsuperscript{105}. Calonysterone (132) has exerted a stronger effect on the Akt phosphorylation in mammalian skeletal muscle cells than the more abundant 20-HE\textsuperscript{105}. Akt is a serine/threonine-kinase which plays multiple key roles in cellular signaling and metabolic processes, such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration. Akt activation followed by elevation in intracellular calcium levels led to an increased protein synthesis in a mouse skeletal muscle myotube cell line C2C12\textsuperscript{164}. Although the mechanism behind PE-mediated activation of Akt is not clear, its downstream effectors represent a major signaling pathway that controls protein turnover and may regulate skeletal muscle activity, which also has been reported for maintaining the aging of muscles\textsuperscript{165}. The metabolism-modulatory and tissue differentiation activities of calonysterone indicate the interconnecting mechanisms of tissue differentiation and growth modulation (Fig. 11).

5.5. Antidiabetic potential

The anabolic modulatory activities of PEs suggest their potential use in diabetic conditions for their blood glucose-lowering properties through the direct stimulation of pancreatic \beta-cells\textsuperscript{165}. PEs extracted from the plant \textit{Ajuga iva} (Lamiaceae) have exhibited \textit{in vivo} potential therapeutic effects against experimental diabetes induced by alloxan\textsuperscript{166}. The extracted PE caused reduction in blood glucose levels in the diabetic rats; it also reduced the levels of blood urea nitrogen, creatinine, tri-glycerides, cholesterol, and lipid peroxidation. These effects were mediated by increasing the levels of antioxidant enzymes, such as catalase, SOD, and glutathione peroxidase activity. Regeneration of islets and reduction of the acinar cells atrophy
was also reported. Another study reported that the administration of PEs in the form of phytoestrogen inhibited glucogenic and lipogenic enzymes and were associated with the regulation of the antioxidant enzymes activity, especially catalase, glutathione peroxidase, and SOD. The decreases of these enzyme levels and activity reductions are known to be essential for the reduced secretion of pancreatic insulin. These reports indicate that PEs may be used for reducing the ROS levels and thus oxidative stress and glucose oxidation, which is a common characteristic of diabetes.

Diabetes is further associated with reduced wound-healing and slow regeneration as a sequential mechanism associated with tissue growth factor. A PE-rich plant, *Stachys hissarica*, has exhibited in vivo wound-healing activity in rats. The wound-healing activity of this plant is more effective than the known drug methyluracil (2,4-dioxo-6-methyl-1,2,3,4-tetrahydropyrimidine), especially in the case of alloxan-induced diabetic animals. These reports are indicative of antidiabetic as well as wound-healing properties of PEs and project their possible applications in pathophysiological conditions of oxidative stress.

5.6. Antimicrobial activities

Antioxidant effects were further reported in association with antimicrobial responses of PEs, such as 22-epi-ajugasterone C, which has shown DPPH radical scavenging activity followed by antimicrobial properties. It showed a weak scavenging effect as compared with myricetin and exhibited antimicrobial properties.
against multi-resistant strains, such as *Staphylococcus aureus*, *Escherichia coli*, *Serratia sp.*, *Klebsiella pneumoniae*, and *Candida albicans*.\(^{68}\) (22R,24R,25S,26S)-2b,3b,14a,20R-Tetrahydroxy-26a-methoxy-6-oxo-stigmast-7-ene-22,26-lactone (149), (22R,24R,25S)-2b,3b,14a,20R,26S-pentahydroxy-6-oxo-stigmast-7-ene-22,26-lactone (150), and (22R,25S)-2b,3b,14a,20R,24S-pentahydroxy-6,26-dioxo-stigmast-7-ene-22,26-lactone (151) also exhibited moderate antibacterial activities against tested oral pathogens.\(^{114}\)

### 5.7. Anticancer activities

Some of the bioactivities of PEs are related to cell proliferation and growth. A known PE Z-24(28)-dehydroamararone B, isolated from the seeds of *L. carthamoides*, has shown potent growth-inhibitory activity against *Drosophila melanogaster* B1 cells with median effective dose (ED\(_{50}\)) of 0.52 \(\mu\)mol/L.\(^{169}\) Ajugacetalsterone E and four other neoclerodane diterpenoids from *A. decumbens* inhibited cell proliferation of the MCF-7, HepG2, A549, and B16 cell lines as an anticancer prospect.\(^{170}\) Two PEs extracted from *Ajuga forrestii* and evaluated for their cytotoxic effects against the A-549 human lung cancer cell, HepG2 human hepatoma cell, MCF-7 human breast cancer cell, and HeLa human cervical carcinoma cell showed no significant inhibitory activities. However, they might interact when used in combination with other extracted PEs. An aqueous extract of *A. forrestii* containing iridoid compounds exhibited potential cytotoxicity with IC\(_{50}\) values ranging between 10 and 19 \(\mu\)mol/L, while selected iridoid glycosides exhibited more significant cytotoxicities with IC\(_{50}\) values ranging between 7 and 14 \(\mu\)mol/L.\(^{170}\) A lipophilic *Leucea* root extract containing the major PE 20-HE demonstrated potent cytotoxic effects on MCF-7 cells. The extract exhibited cell proliferation with IC\(_{50}\) 30 \(\mu\)g/mL yet 20-HE alone was not as effective.\(^{171}\) Genome-wide expression analysis for differential transcriptional expression of 241 genes showed that *Leucea* extract treatment was comparatively more effective that treatment with tamoxifen, a commonly used medication for preventing breast cancer in women.\(^{172}\) The cytotoxic potentials of \(n\)-butanol fraction of *Ajuga chamaecestus*, which mainly contains melilotoside, phenylethyl glycosides, and PEs, were assessed against human cancer cell lines (T47D, HT-29, and Caco-2) and the normal cell line (NIH 3T3) showed no cytotoxicity even up to 400 \(\mu\)g/mL concentration of the extract.\(^{172}\) Additionally, a previous study using three major compounds (20-HE, cyasterone, and \(\delta\)-acetylharpagine) from the diethyl ether fraction of *A. chamaecistus* showed inactivity in the cytotoxicity evaluation.\(^{172}\)

The ABCB1 transporter is a type of efflux pump that plays a key role in the chemo-resistance of various tumors and particularly of cancer stem cells. Fifty-eight ecdysteroids were tested for their activity against L5178 mouse T-cell lymphoma cells and their subcell line transfected with pHa MDR1/A retrovirus over-expressing the human ABCB1 efflux pump.\(^{173}\) The tested PEs showed slight inhibition of cell proliferation, but notable modulation of the efflux of rhodamine 123 mediated by the ABCB1 transporter.\(^{174}\) Screened PEs showed mild to strong synergism or antagonism when tested in combination with doxorubicin, which suggests that ecdysteroids could be potentially used for enhancing the specific structure–activity relationships for cancer chemotherapeutics. Another study analysed the cytotoxic effects of a preparation of three fluorinated derivatives of PEs on the ABCB1 efflux transporter expressing four human breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-361 and T47D), a neuroblastoma cell line (SH-SY5Y), and a mouse lymphoma cell line (L5178) by MITT assay.\(^{174}\) Fluorinated PE derivatives increased the ABCB1 inhibitory effect and caused inhibition of cell proliferation. Also, the derivative compound 5 (14,25-difluoro analog) exerted higher chemo-sensitizing activity to doxorubicin as compared to its parental compound.\(^{175}\) Seeing the importance of ABCB1 efflux pump transporters in cancer chemo-resistance and stem cell properties, PE derivatives could be potentially used in sensitizing ABCB1 over-expressing cancer cells for enhanced chemotherapeutic potentials.

An interesting study tested the combinatorial effects of 20-HE with half doses of cisplatin and adriamycin combination on the development of subcutaneously and intraperitoneally transplanted P388 and L1210 leukemia and metastasizing B16 melanoma.\(^{173}\) 20-HE significantly stimulated the chemotherapeutic effects at low doses via a mechanism involving of cytostatic effects, resulting in tumor growth and an increase in the survival rate and lifespans of mice. Also, the 20-HE showed a comparably improved antimetastatic activity index at high doses of the antitumor drugs in mice. Mechanistically, it was demonstrated that the low doses of cisplatin and edysterone affected the biochemical dynamics of cells as well as altering the protein and DNA biosynthesis in the liver, pancreas, thymus, spleen, and adrenals of tumor-bearing mice. The combination of cisplatin with higher doses of 20-HE could affect the metastatic response.\(^{175}\) These observations lead to the hypothesis that these doses of PEs can influence the therapeutic index, which would warrant considerable attention in the processes of drug development and disease management. The plausible interconnecting pathways involving oxidative stress and free radicals, apoptosis, and cell cycle regulation are depicted in Fig. 12. These observations additionally suggest that PEs can influence the therapeutic index of chemotherapeutic agents by modulating cell proliferation mechanism and inducing apoptosis, which fetches considerable attention in the process of drug development and disease management.

### 6. Molecular mechanisms of action of phytoecdysteroids

The first characterized ecdysteroid, edesone, was isolated from the silkworm *Bombyx mori*. In *Spinacia oleracea* L. (spinach), the major ecdysteroid is edesone, which can occur in concentrations between 50 and 800 \(\mu\)g per g fresh weight tissue, depending on growth rate of the plant.\(^{176}\) Numerous studies have described a variety of pharmacological effects of ecdysteroids, such as an increase in carbohydrate and fatty acid metabolism, stimulation of immune response, and an increase in protein synthesis and general health-promoting activity.\(^{177}\)

The bioactivity of PE and molecular mechanisms are not well characterized. In arthropods, the effects of PEs are mediated through nuclear receptor complexes consisting of two proteins, which are ecdysterone and ultraspiracle protein. In mammals, PEs execute their effects through human steroid hormone receptors as the nuclear receptor complex is not expressed in mammals. Besides that, no significant binding affinity of PEs to the androgen receptors (ARs) has been observed.\(^{173}\) Alternatively, it was hypothesized that PEs interact with receptors located in the cell membrane of mammalian cells. The antioxidant effects of 20-HE on rat liver mitochondrial fractions were analyzed, and showed that it intervenes with the liposomal membrane oxidation.\(^{78}\) The rate of free-radical formation was reduced by 20-HE in cholesterol-dependent manner, while \(\alpha\)-tocopherol showed lower antioxidant
effect in the membrane. 20-HE showed potent antioxidant action in combination with \(\alpha\)-tocopherol in membranes that contain excess cholesterol, which indicates the free radical scavenging mechanism\(^{178}\). The treatment of mouse skeletal muscle cell line C2C12 (murine myotubes and human primary myotubes) with PE demonstrated an increase in protein synthesis activity by up to 20\%\(^{157,164}\). Furthermore, it strengthened the gripping capacity in rats, which is a health promoting activity. These effects were observed to be mediated via inhibition of the phosphoinositide kinase-3 (PI3K) in skeletal muscle cells; the PI3K pathway activates Akt which was shown to increase protein synthesis in skeletal muscles C2C12 myotubes when treated with 20-HE\(^{164}\).

Growing evidence suggests that besides androgens and insulin-like growth factor 1, estrogens are also involved in the regulation of skeletal muscle homeostasis. Estrogen receptors (ERs) are ligand-activated transcription factors belonging to the family of nuclear receptors\(^{179}\). With respect to molecular mechanisms mediating the anabolic effects of PEs, it has been demonstrated that selective activation of ERs strongly induces regeneration and most likely induces de novo fibre formation in toxin-damaged muscles of female rats\(^{180}\). These observations indicate a major role of ERs in skeletal muscle homeostasis in both genders. Studies on animal models also have a focus of identifying the potential role of PEs on ERs.

In addition to classical nuclear receptor responses, mammalian steroid hormones that are structurally related to PEs have been known to elicit rapid non-genomic signaling events that mediate cell proliferation and survival. These responses may involve alterations in secondary signals, such as Ca\(^{2+}\) or cyclic adenosine monophosphate\(^{181}\). Mammalian steroid hormones increase Ca\(^{2+}\) influx in a variety of cell types, including cardiac myocytes and skeletal muscle\(^{182,183}\). Several of these effects have been attributed to a new role for already identified nuclear receptors, such as the ERs and ARs\(^{13}\). Thus, PEs may be considered as putative target molecules of ER and AR for their respective signaling processes of anabolic function, protective effects, and anti-inflammatory effects.

The anticancer effects of a lipophilic Leuzea root extract containing 20-HE was found to be mediated via gene regulation in an antiproliferative manner through downregulation of cyclin E2 (CCNE2), forkhead box M1 (FOXM1), G-2 and S-phase expressed 1 (GTSE1), proliferating cell nuclear antigen (PCNA), induced expression of cyclin G2 (CCNG2), growth arrest and DNA-damage-inducible, alpha (GADD45\(\alpha\)) and tumor protein p53 inducible nuclear protein 1 (TP53INP1) pointed to cell cycle arrest at the G1/S-transition checkpoint in human breast adenocarcinoma cells MCF-7\(^{171}\). In addition, approximately thirty genes associated with DNA replication and synthesis appeared to be downregulated by the extract, which indicated that reduced replication rate and cell cycle arrest at the G1/S-transition checkpoint is more specific than the molecule mechanism in MCF-7 cells\(^{171}\). The comprehensive miscellaneous biological effects of PEs corroborate with the interconnective mechanisms of tissue differentiation and the growth modulation, apoptosis, cell proliferation, and modulation of glycolytic metabolism (Fig. 11). These intercorrelated mechanisms affect the acute and chronic pathophysiological conditions of oxidative stress and inflammation, especially diabetes and cancer.

7. Conclusions and future directions

In this review, we have summarized the isolation and characteristics of 212 new PEs from different plant species, reported between 1999 and 2019. The diverse bioactivities that were reported for these PEs include antioxidant, anti-inflammatory, antimicrobial, analgesic, anabolic, adaptogenic, hepatoprotective, antidiabetic, and anticancer properties (Fig. 13). In addition to this, we have discussed various mechanisms by which several PEs exert their biological actions. However, despite the copious amount of PE research that has been performed over the past two decades, most of the results cited in the current review are based on in vitro studies; data associated with in vivo and clinical studies are very limited. The versatile and potent pharmacological properties of

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**Figure 12** Proposed mechanism of the anticancer effects of phytoecdysteroids through induction of cell death. Phytoecdysteroids interact through the ABCB1 transmembrane protein and activates mitochondrial apoptosis pathway as well as it suppresses oxidative stress and causes cell cycle arrest. These collectively lead to inhibition of cell proliferation and cell death. ABCB1, transmembrane efflux transporter; CAT, catalase; PARP, poly(ADP-ribose) polymerase; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.
this group of phytochemicals allow them to be of considerable interest, especially for their antioxidant, anti-inflammatory, and growth-regulatory activities. This further adds to their scientific characterization and specific bioactivity. The function of ABCB1 efflux pump transporters was limited by the usage of PE derivatives, which then leads to the inhibition of the proliferation of cancer cells. As ABCB1 are essentially involved in cancer chemoresistance and the stemness of cancer cells, PE derivatives appear to be potential target compounds in the chemotherapeutic drug development process. Also, PEs derivatives have the potential to enhance the therapeutic efficacy of chemotherapeutics agents, especially tamoxifen, cisplatin and doxorubicine, in a synergistic or agonistic manner in several types of cancer cells. PE calonysterone appears to modulate Akt signaling, which further correlates with various essential cell growth signaling processes, such as glycolytic metabolism, cell proliferation, apoptosis, cell migration, and tissue differentiation. The antioxidant, anti-inflammatory, and growth-modulatory properties of PEs represent a considerable benefit of using them in therapy and in the prevention of human diseases. As the two conditions are essentially associated with carcinogenesis, PEs can also be utilized to manage not only oxidative stress and inflammation, but also to limit the carcinogenic process through their usage. This review discusses the promising biological and pharmacological properties of various PE-rich plant extracts, which may be utilized in the development of nutraceutical and pharmaceutical products after further confirmatory research on their efficacy and safety.

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Author contributions

Niranjan Das conceived the study and prepared the manuscript draft. Siddhartha Kumar Mishra and Eunjus S. Ali analysed the contents and assisted in the finalization of the manuscript draft. Anusha Bishayee improved the language of the manuscript. Anupam Bishayee supervised the entire project and edited the manuscript with suggestions for improvements. All the authors made conceptual contributions to the content and have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

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