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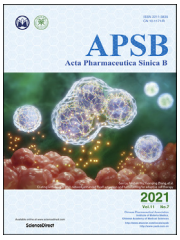

REVIEW

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

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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 β-side chain at carbon-17. The essential characteristics of ecdysteroids are 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. 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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1. Introduction

Phytoecdysteroids (PEs) are a class of biologically-active chemicals that plants 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 ecdysone, 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 zooecdysteroids 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 zooecdysteroids. Several common ecdysteroids, such as ecdysone (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 ~6% of all plants contain detectable levels of ecdysteroids[6]. 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[6]. 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[7–9]. These chemicals are also able to alter and switch genes around in plants through DNA binding[10,11]. Even in mammals, these compounds have exhibited unique biological results, such as a stimulatory effect on growth and metabolism[12], and an effect on anabolic and steroidal growth regulations[13]. A recent study reports that a mixture of PEs extracted from *Serratula coronata* enhances the productivity and vitality of ducklings, which suggests the existence of PEs' growth and physiologically favoring properties[14]. For this reason, these compounds have been reported to be used by athletes and sports personnel as stimulants in moderately low dosages[15]. 20-HE was shown to have influences on the sexual activity of male rats[16]. Physiological and pharmacological studies indicate that ecdysteroids contribute to an increase in protein synthesis in body builders, patients with acquired immunodeficiency syndrome, and patients with cancer[17]. Ecdysteroids also have shown effects similar to those of antidepressants, shielding the body from stress and improving physical and sexual performances[18]. A recent study reported five PEs from ethyl acetate and *n*-butanol fractions of *Sphenocentrum jollyanum* that had shown urease inhibitory and antacid activities, suggesting their potential usage in ulcerative colitis[19]. Because of such claimed multifactorial activities[20], 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[21]. PEs have been utilized by sportsmen and bodybuilders for improving physical performance, and for enhancing stress resistance by promoting vitality[22]. Different PEs could show binding interactions with human nuclear receptors[22]. The PE derivatives displayed hormonal effects on invertebrates and exerted several favorable, non-hormonal, biological effects on mammals[23]. 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 nesecciates 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 phytoecdcysteroids 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://ecdybase.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, ecdysones, secondary metabolites, phytoinsecticides, antioxidant, anti-

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 C_{27}, C_{28}, or C_{29} 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[26]. The mammalian steroid hormones have more variable structures and they generally lack the polyhydroxylated side chain characteristic of ecdysteroids; accordingly, they are quite nonpolar[26]. 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[27]. So far, more than 200 PEs, mostly from genus *Ajuga*, *Podocarpus*, *Polypodium*, and *Silene*, have been reported[6].

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.[28,29] 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*[30]. 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 phytoecdcysteroids (**5**−**216**) with their plant sources is presented in Supporting Information Table S1. The chemical structures of new PEs isolated from 18 plant families, such as Amaranthaceae, Asteraceae, Blechnaceae, Caryophyllaceae, Clavicipitaceae, Commelinaceae, Dioscoreaceae, Gleicheniaceae, Lamiaceae, Liliaceae, Limnanthaceae, Lygodiaceae, Malvaceae, Menispermaceae, Polypodiaceae, Polyporaceae, Rhodomelaceae, and Taxaceae, are illustrated in Figs. 2–8.

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[5,31,32].

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 F_{254} 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[33]. The details of chromatographic procedures for separation of PEs were previously reviewed[34].

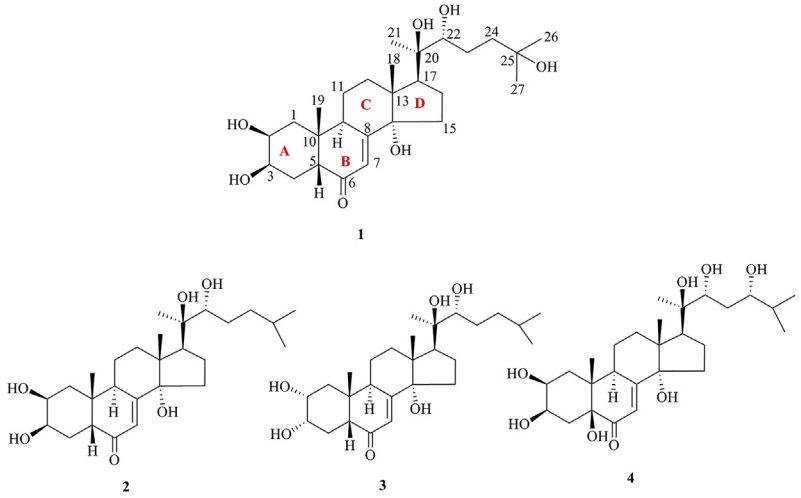

Figure 1 Structures of 20-hydroxyecdysone (**1**), ponasterones A (**2**), B (**3**) and C (**4**).

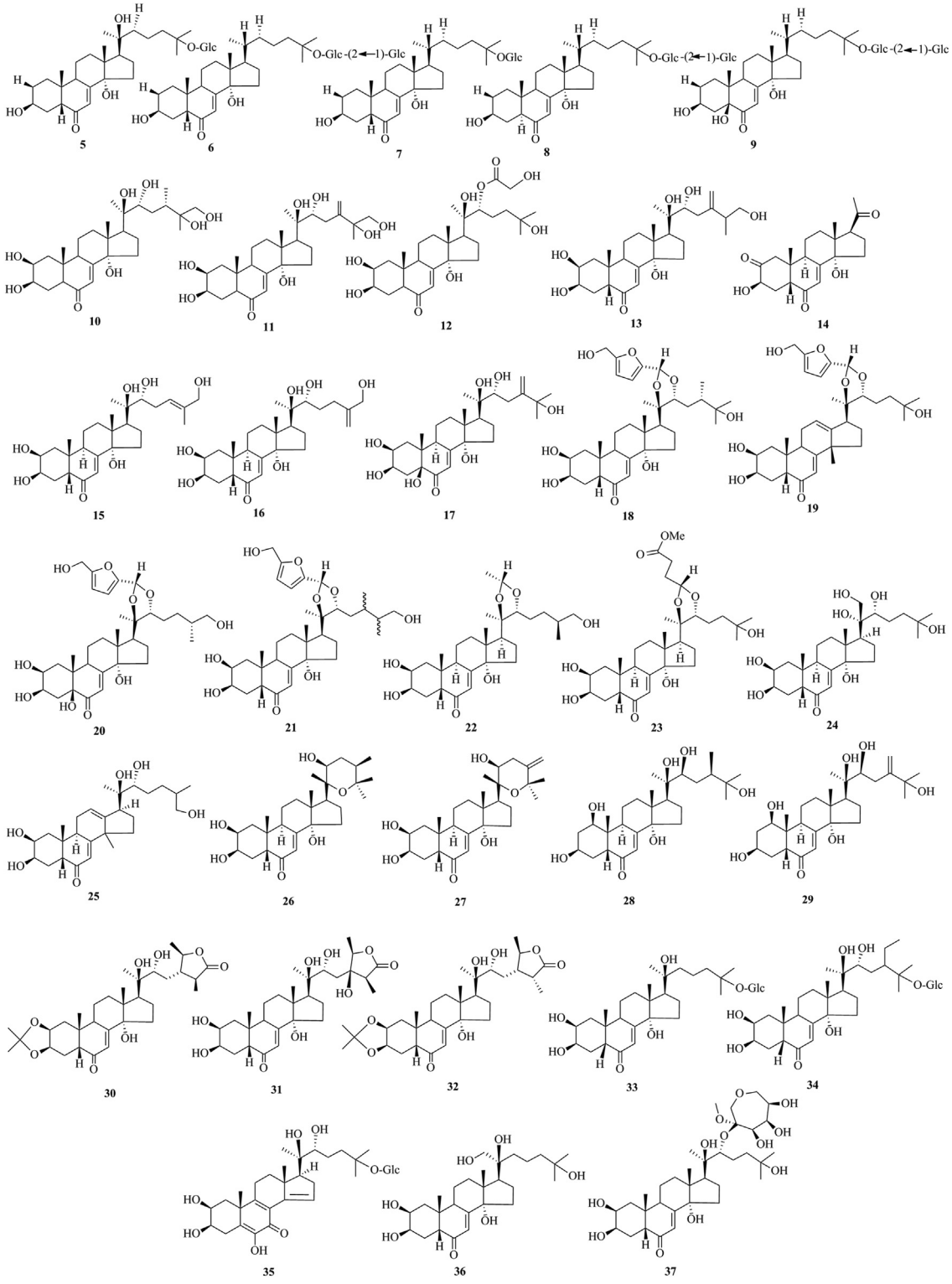

Figure 2 Structures of reported new phytoecdysteroids (**5**−**37**) from Amaranthaceae family.

3.2. Identification of phytoecdysteroids

The 14α-hydroxy-7-en-6-one chromophore of PEs showed characteristic ultra-violet absorption in methanol (MeOH) at λ_{max} 240–245 nm[35]. 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

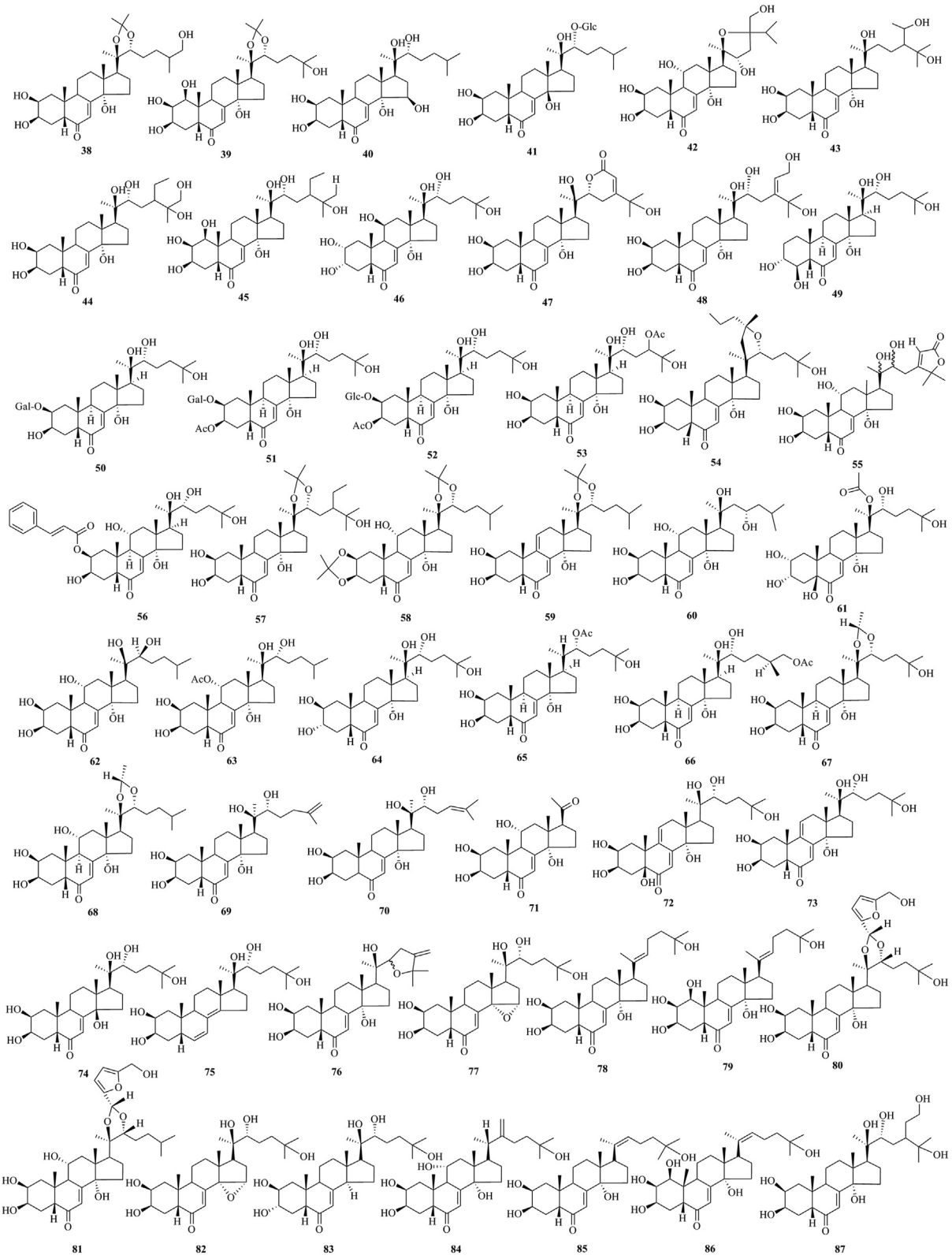

Figure 3 Structures of reported new phytoecdysteroids (**38**−**87**) from Asteraceae family.

resonances of C-6 and C-20 appear to be the lowest at δ_C 201–204 and 207–209 ppm. The carbon resonances for C-2 and C-3 are near and δ_C 67–69 and C-14's carbon resonances are near δ_C 83–85 ppm. Additionally, the carbon resonance values of C-7 and C-8 appear at δ_C 121–123 and 162–165 ppm. The ^{13}C-NMR spectra of some PEs have already been reviewed[36]. However, it is necessary to include that therea 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 isopropylidene 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[37]. 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 glyceryladehyde-3-phosphate also *via* the non-mevalonate pathway[32]. 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[32]. 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-D-xylulose could be used as an intermediate in mevalonate-independent deoxy-xylulose phosphate pathway (DOXP). *Polypodium vulgare* 20-HE might be synthesized from a Δ7 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 *Taxas 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 3α- and 4β-positions to C-4 and C-5 by concomitant 1,2-Wagner-Meerwein hydride migrations from the 4β-to the 5β- and from the 3α-to the 4α-position. The biosynthetic pathway study by Hyodo and Fujimoto[40] in *Ajaga reptans* hairy roots indicated that 3β-hydroxy 5β-cholestan-6-one is converted into 2β,3β-dihydroxy 5β-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 phytoecdcysteroids reported from 17 plant families from 1999 to 2019, along with various bioactivities of PEs. The new phytoecdcysteroids 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, Gleicheniaceae, Lamiaceae, Liliaceae, Limnanthaceae, Lygodiaceae, Malvaceae, Menispermaceae, Polypodiaceae, Polyporaceae, 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 Gleicheniaceae 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 Polyporaceae 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).

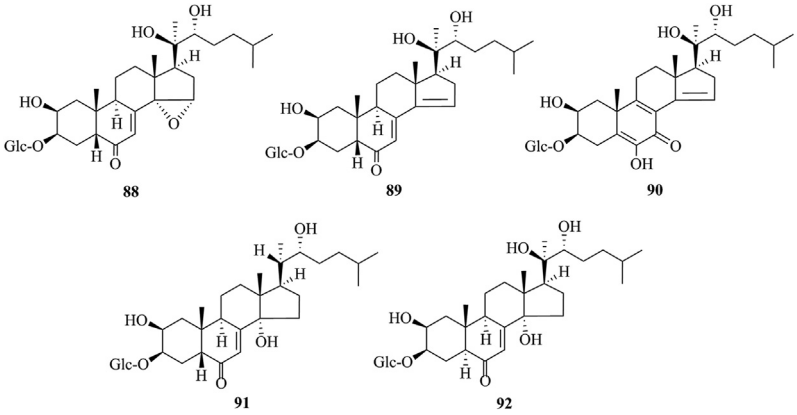

Figure 4 Structures of reported new phytoecdysteroids (**88**−**92**) from Blechnaceae family.

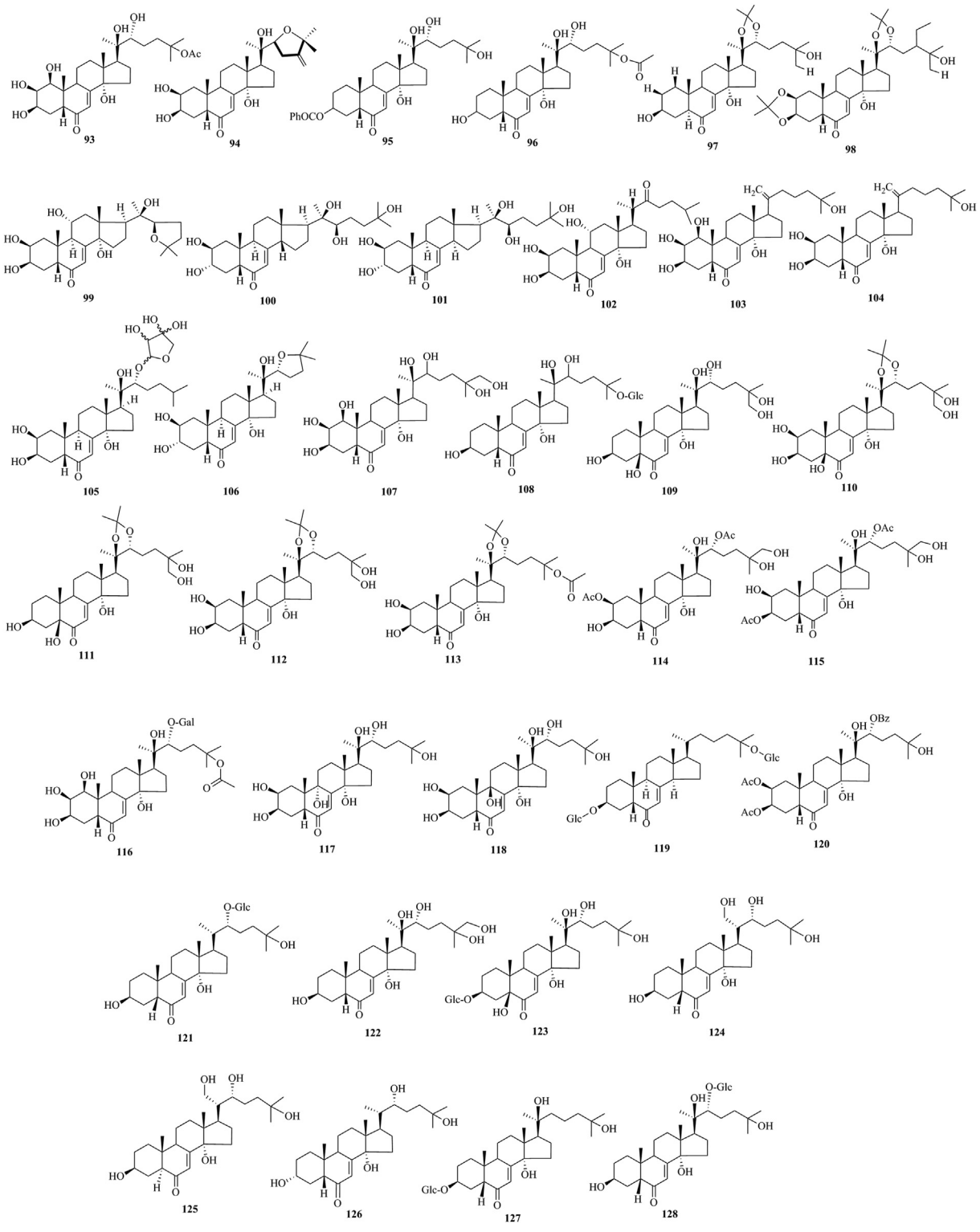

Figure 5 Structures of reported new phytoecdysteroids (**93**−**128**) from Caryophyllaceae family.

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-*O*-*β*-D-glucopyranoside (**5**), 2,22-dideoxyecdysone 25-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**6**), 2,22-deoxyecdysone 25-*O*-*β*-D-glucopyranoside (**7**), (5*α*)-2,22-dideoxyecdysone 25-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**8**), 2,22-dideoxy-5*β*-hydroxyecdysone 25-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**9**), isolated from the plant *Froelichia floridana*[41], 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*[42,43], 3*β*,14*α*-

dihydroxy-5β-pregn-7-ene-2,6,20-trione (**14**), 24,25-dehy-droinokosterone (**15**), 25,27-dehydroinokosterone (**16**), 5β-hydroxy-24(28)-dehydromakisterone A (**17**), isolated from *Chenopodium album* Willd[44,45], niuxixinsterone A [(20*R*,22*R*,24*S*)-20-*O*,22-*O*-(5′-hydroxymethyl)-furfurylidene-2β,3β,14α,25-tetrahydroxy-5β-ergost-7-en-6-one] (**18**), niuxixinsterone B [(20*R*,22*R*)-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 [(20*R*,22*R*,25*R*)-20-*O*,22-*O*-(5′-hydroxymethyl)-furfurylidene-2β,3β,5β,14α,26-pentahydroxycholest-7-en-6-one] (**20**)[46], niuxixinsterone D (**21**)[47], (25*S*)-20,22-*O*-(*R*-ethylidene) inokosterone (**22**), 20,22-*O*-(*R*-3-methoxycarbonyl) propylidene-20-hydroxyecdysone (**23**)[48], achyranthesterone A [2β,3β,14α,20*S*,21,22*R*,25-heptahydroxycholest-7-en-6-one] (**24**)[49], (20*R*,22*R*)-2β,3β,20,22,26-pentahydroxy-cholestan-7,12-dien-6-one (**25**)[50], isolated from the plant *Achyranthes bidentata*[50], 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*[51], 2,3-isopropylidene cyasterone (**30**), 24-hydroxycyasterone (**31**), 2,3-isopropylidene isocyasterone (**32**), isolated from *Cyathula officinalis* Kuan[52], pfaffiaglycosides C (**33**),

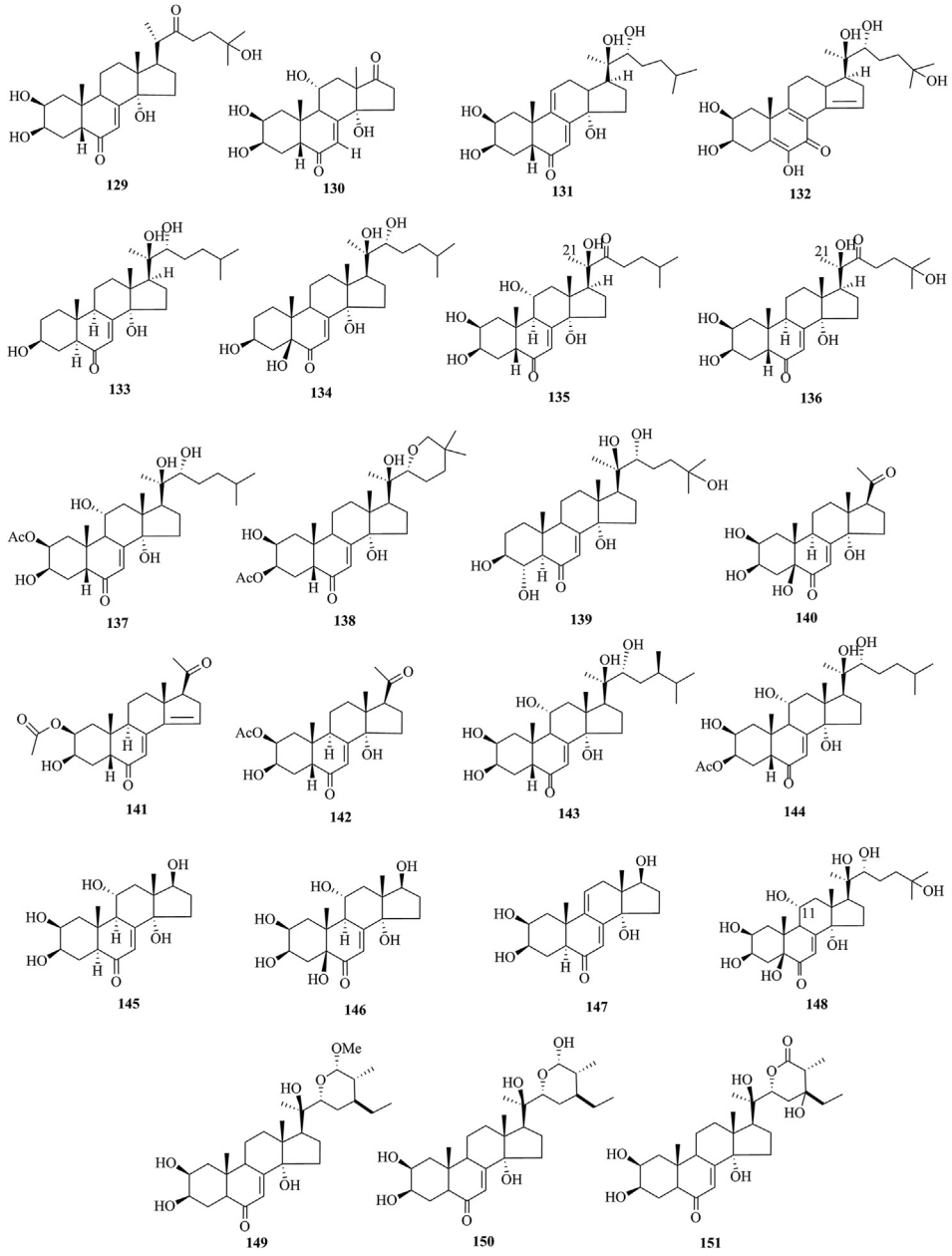

Figure 6 Structures of reported new phytoecdysteroids (**129**−**151**) from Clavicipitaceae, Commelinaceae, Dioscoreaceae, and Gleicheniaceae families.

pfaffiaglycosides D (**34**), pfaffiaglycosides E (**35**), isolated from *Pfaffia glomerata*[53], (20*R*)-22-deoxy-20,21-dihydroxyecdysone (**36**), isolated from *Rhagodia baccata* (Labill.) Moq.[54], and septanoecdysone (**37**), isolated from the plant *Atriplex portulacoides* L.[55].

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

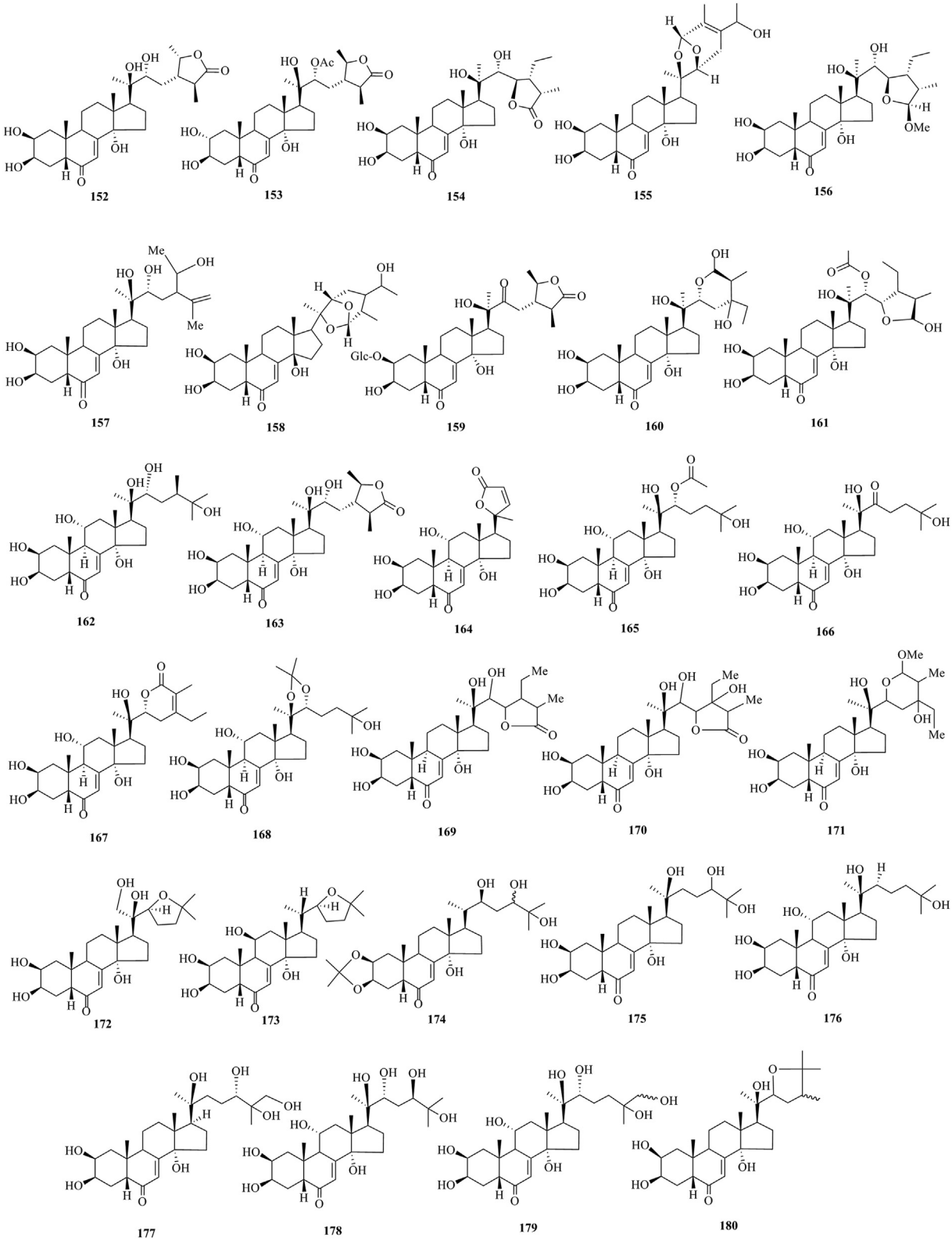

Figure 7 Structures of reported new phytoecdysteroids (**152**−**180**) from Lamiaceae family.

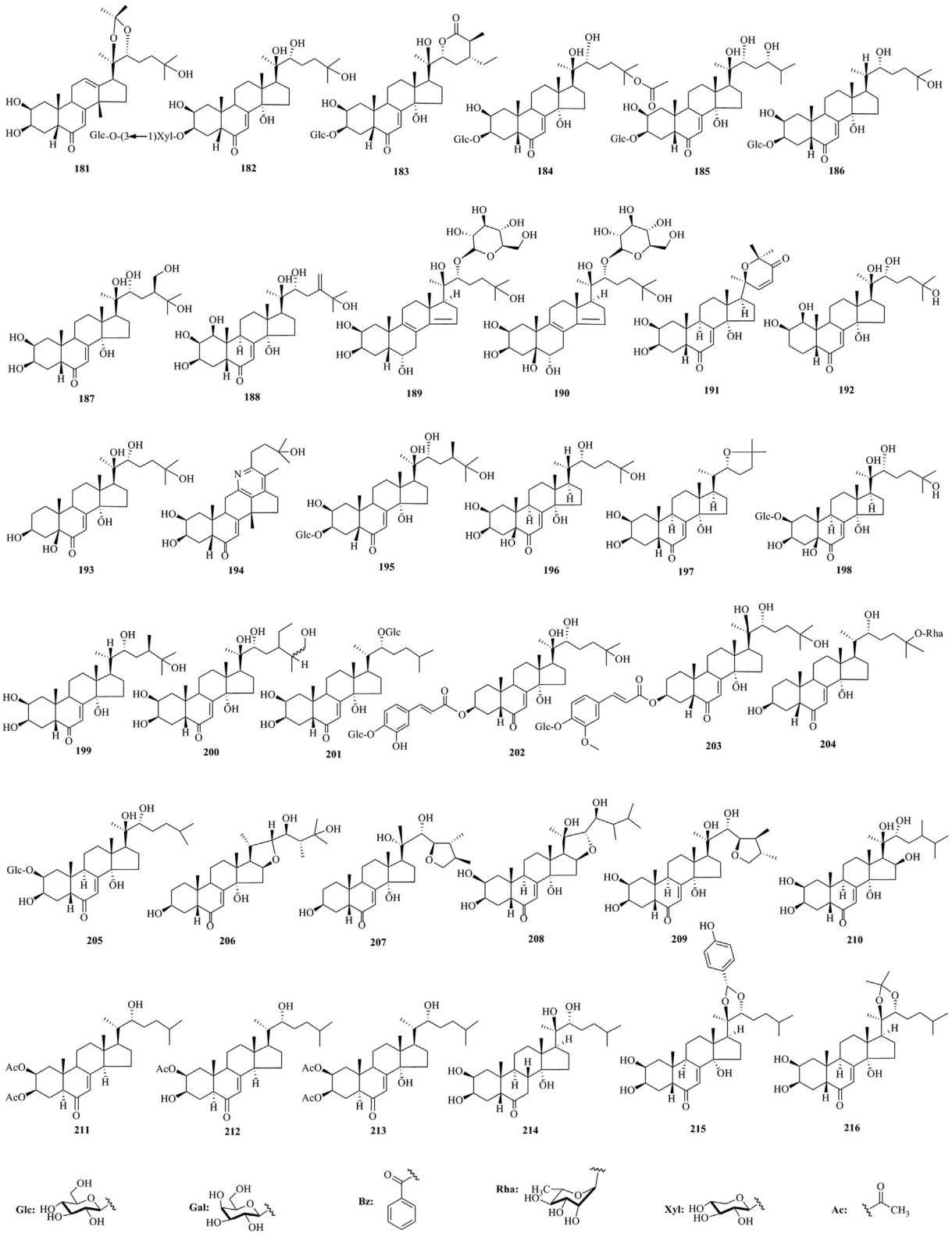

Figure 8 Structures of reported new phytoecdysteroids (**181**−**216**) from Liliaceae, Limnanthaceae, Lygodiaceae, Malvaceae, Menispermaceae, Polypodiaceae, Polyporaceae, Rhodomelaceae, and Taxaceae families.

(**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,20*R*,22*R*,25-heptahydroxy-6-one] (**46**), isolated from the plant *L. carthamoides*[57], leuzeasterone (**47**) and (24*Z*)-29-hydroxy-24(28)-dehydromakisterone C (**48**) isolated

from the plant *L. carthamoides*[58], coronatasterone (2-deoxy-3-*epi*-4β,20-dihydroxyecdysone) [(20*R*,22*R*)-3α,4β,14α,20,22,25-hexahydroxy-5β-cholest-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,20ε,22ε-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-diacetonide (**58**) and 5-deoxykaladasterone-20,22-monoacetonide (**59**), isolated from *R. uniflorum*[65], uniflorsterone (**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*[68], ajugasterone 11-acetate (**63**)[69], 3-*epi*-20-hydroxyecdysone (**64**)[70], ecdysone 22-acetate (**65**), (25*S*)-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 stachysterone C (**70**), isolated from *Klaseopsis chinensis*[72], 11α-hydroxypoststerone (**71**)[73], herkesterone [5β,25-dihydroxydacryhainansterone] (**72**)[73], 25-hydroxydacryhainansterone (**73**)[74], 14-*epi*-20-hydroxyecdysone (**74**)[74], 2β,3β,20*R*,22*R*,25-pentahydroxy-5β-cholest-6,8(14)-dien (**75**)[75], 24-methylene-shidasterone (**76**)[75], 14α,15α-epoxy-14,15-dihydrostachysterone B (**77**)[75], 20,22-didehydro taxisterone (**78**)[76], 1-hydroxy-20,22-didehydrotaxisterone (**79**)[76], serfurosterone A (**80**)[77], serfurosterone B (**81**)[77],14,15α-epoxy-(20*R*,22*R*)-2β,3β,20,22,25-pentahydroxy-5β-cholesta-7,14-dien-6-one (**82**)[78], (20*R*,22*R*)-2β,3α,20,22,25-pentahydroxy-5β-cholesta-7-en-6-one (**83**)[78], 22-methylene-2β,3β,11α,14α,25-pentahydroxy-5β-cholesta-7-en-6-one (**84**)[78], 2β,3β,14α,25-tetrahydroxy-5β-cholesta-7,20(22)-dien-6-one (**85**)[78] and 1β,2β,3β,14α,25-pentahydroxy-

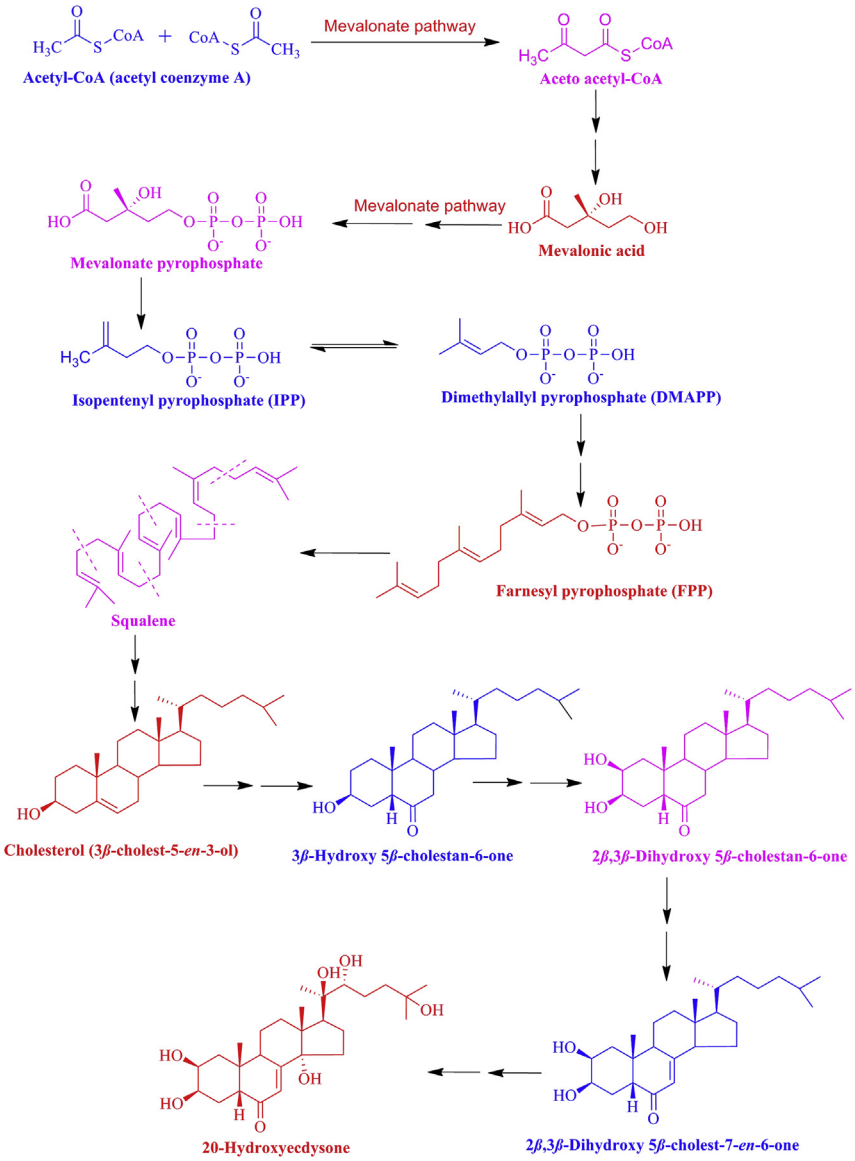

Figure 9 Probable biosynthetic route of 20-hydroxyecdysone in plants.

5β-cholesta-7,20(22)-dien-6-one (**86**)[78], isolated from *Serratula wolffii*, and (24*R*)-24-(2-hydroxyethyl)-20-hydroxyecdysone (**87**) isolated from the plant *Serratula strangulate*[79].

4.3. Blechnaceae

The Literature survey indicates that five new ecdysteroids have been reported from the Blechnaceae family. These are brainesteroside A [14-deoxy-14α-15α-epoxyponasteroside A] (**88**), brainesteroside B [14-deoxy-14,15-didehydroponasteroside A] (**89**), brainesteroside C [25-deoxycalonysterone-3-*O*-β-D-glucopyranoside] (**90**), brainesteroside D [25-deoxyecdysone-3-*O*-β-D-glucopyranoside] (**91**), and brainesteroside E [5-*epi*-ponasteroside A] (**92**) isolated from the plant *Brainea insignis*[80].

4.4. Caryophyllaceae

Thirty-six new ecdysteroids have been reported from the Caryophyllaceae family. The new ecdysteroids are integristerone A 25-acetate (**93**), isolated from *Silene brahuica*[81], japonicone (22,25-epoxy-24-methylene-2,3,14,20-tetrahydroxycholest-7-en-6-one) (**94**), isolated from *Sagina japonica*[82], 2-dehydroxyecdysterone-3-*O*-benzoate (**95**)[83], 2-deoxyecdysterone-25-acetate (**96**)[84], isolated from *Silene wallichiana*, 5α-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**97**)[85], makisterone C 2,3; 20,22-diacetonide (**98**)[85] isolated from *Silene viridiflora*, (11α)-11-hydroxyshidasterone [(2β,3β,5β,11α,22*R*)-22,25-epoxy-2,3,11,14,20-pentahydroxy cholest-7-en-6-one] (**99**), (2β,3β,5β,14β,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**100**), (2β,3α,5β,14α,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**101**), 22-dehydro-20-deoxy ajugasterone C (**102**), 1-hydroxy-22-deoxy-20,21-didehydro ecdysone (**103**), 22-deoxy-20,21-didehydro ecdysone (**104**), ponasterone A-22-apioside (2β,3β,14α,20*R*-tetrahydroxy-22-{[3,4-dihydroxy-4-(hydroxymethyl)-tetrahydrofuran-2-yl]oxy}-5β-cholest-7-en-6-one) (**105**), 3-*epi*-shidasterone [22,25-epoxy-2β,3α,14α,20*R*)-tetrahydroxy-5β-cholest-7-en-6-one] (**106**), isolated from *S. wolffii*[86–88], 26-hydroxyintegristerone A (**107**), isolated from *Silene frivaldszkyana*[89], 2-deoxy-20-hydroxyecdysone 25-glucoside (**108**), isolated from *Silene gigantean*[89], 2-deoxy-5,20,26-trihydroxy ecdysone (**109**), 5,20,26-trihydroxyecdysone 20,22-acetonide (**110**), 2-deoxy-5,20,26-trihydroxyecdysone 20,22-acetonide (**111**), 20,26-dihydroxyecdysone 20,22-acetonide (**112**), 20-hydroxyecdysone 20,22-monoacetonide-25-acetate (**113**), 2,22-diacetate-20,26-dihydroxyecdysone (**114**), 3,22-diacetate-20,26-dihydroxyecdysone (**115**), isolated from *S. viridiflora*[90–92], 22-*O*-α-D-galactosylintegristerone A 25-acetate (sileneoside H) (**116**), isolated from *S. brahuica*[93], 9α,20-dihydroxyecdysone (**117**), 9β,20-dihydroxyecdysone (**118**), isolated from *Silene italic* ssp. *nemoralis*[94,95], 3-*O*-β-D-glucopyranosyl-3β,25-dihydroxy-5β-cholest-7-en-6-one-25-*O*-β-D-glucopyranoside (**119**), isolated from *Silene montbretiana*[96], 2,3-diacetate-22-benzoate-20-hydroxyecdysone (**120**), isolated from *Silene guntensis* B. Fedtsch[97], 2-deoxyecdysone 22β-D-glucoside (**121**), 2-deoxy-20,26-dihydroxyecdysone (**122**) and 2-deoxypolypodine B 3β-D-glucoside (**123**), isolated from *Silene pseudotites*[98], 2-deoxy-21-hydroxyecdysone (**124**) and 5α-2-deoxy-21-hydroxyecdysone (**125**), isolated from *Silene otites*[99], 3α,14α,22*R*,25-tetrahydroxy-5β(H)-cholest-7-en-6-one (**126**), isolated from *Acanthophyllum gypsophiloides*[100], and 2,22-dideoxy-20-hydroxyecdysone 3β-*O*-β-D-glucopyranoside (**127**) isolated from *Cucubalus baccifer*[101] and 2-deoxy-20-hydroxyecdysone-22-*O*-β-D-glucopyranoside (**128**), isolated from the plant *Silene italica* ssp. *nemoralis*[102].

4.5. Clavicipitaceae

One new ecdysteroid has been reported from the Clavicipitaceae family. The new ecdysteroid 22-dehydroecdysone (**129**) is isolated from *Nomuraea rileyi* (fungus)[103].

4.6. Commelinaceae

Eighteen ecdysteroids have been isolated from the Commelinaceae family. These ecdysteroids are 11α-hydroxyrubrosterone (**130**)[104], dacryhainansterone (**131**)[105], calonysterone (**132**)[105], cyanosterone A (**133**)[106], cyanosterone B (**134**)[107], 22-oxo-ajugasterone C (**135**)[108], 22-oxo-20-hydroxyecdysone (**136**)[108], ajugasterone C 2-acetate (**137**)[109], shidasterone3-acetate (**138**)[109], isolated from the plant *Cyanotisar achnoidea*, 3β,4α,14α,20*R*,22*R*,25-hexahydroxy-5α-cholest-7-en-6-one (**139**), isolated from the plant *C. achnoidea* C. B. Clarke[110], 5β-hydroxypoststerone (**140**), 14,15-dehydropoststerone 2-acetate (**141**), poststerone2-acetate (**142**), 24-*epi*-atrotosterone A (**143**), ajugasterone C 3-acetate (**144**), isolated from the plant *Cyanotis longifolia*[111], callecdysterol A (**145**), callecdysterol B (**146**), and callecdysterol C (**147**), isolated from the plant *Callisia fragrans*[112].

4.7. Dioscoreaceae

The conducted literature survey indicats that one new ecdysteroid, namely (20*R*)-5β-11α,20-trihydroxyecdysone (**148**), has been isolated from the plant *Dioscorea dumetorum* (Dioscoreaceae)[113].

4.8. Gleicheniaceae

The literature survey indicates that three new ecdysteroids, (22*R*,24*R*,25*S*,26*S*)-2β,3β,14α,20*R*-tetrahydroxy-26α-methoxy-6-oxo-stigmast-7-ene-22,26-lactone (**149**), (22*R*,24*R*,25*S*)-2β,3β,14α,20*R*,26*S*-pentahydroxy-6-oxo-stigmast-7-ene-22,26-lactone (**150**), and (22*R*,25*S*)-2β,3β,14α,20*R*,24*S*-pentahydroxy-6,26-dioxo-stigmast-7-ene-22,26-lactone (**151**), have been isolated from the plant *Diplopterygium rufopilosum* (Gleicheniaceae)[114].

4.9. Lamiaceae

A literature survey indicates that 29 new ecdysteroids have been reported from the Lamiaceae family. These are 28-*epi*-cyasterone [(22*R*,24*S*,25*S*,28*S*) 5β-stigmast-7-ene-26-oic acid, 2β,3β,14,20,22,28-hexahydroxy-6-oxo-γ-lactone] (**152**), isolated from *Eriophyton wallchii*[115], ajugalide-E (**153**) from *Ajuga taiwanensis*[116], breviflorasterone (**154**), ajugacetalsterone C (**155**), ajugacetalsterone D (**156**) from *Ajuga macrosperma* var. *breviflora*[117], decumbesterone A (**157**)[118] and ajugacetalsterone E (**158**)[119] isolated from *Ajuga decumbens*, 22-dehydrocyasterone-2-glucoside (**159**), ajugacetalsterone A (**160**) and ajugacetalsterone B (**161**), isolated from *Ajuga nipponensis*[120], 25-hydroxy-atrotosterone A (**162**), 11-hydroxy-cyasterone (**163**), 11-hydroxy-sidisterone (**164**), turkesterone 22-acetate (**165**), 22 oxo-turkesterone (**166**), 11-hydroxy-Δ24-capitasterone (**167**) and turkesterone 20,22-acetonide (**168**), isolated from *Ajuga turkestanica*[121], reptanslactone A (**169**), reptanslactone B (**170**) and sendreisterone (**171**), isolated from *Ajuga reptans* var. *reptans*[122], 21-hydroxyshidasterone (**172**), 11β-hydroxy-20-deoxyshidasterone (**173**) and 2,3-acetonide-24-hydroxyecdysone (**174**), isolated from *Vitex doniana*[123], 24-*epi*-pinnatasterone (**175**)[124] and scabrasterone (**176**)[124], isolated from *Vitex scabra*, 26-

hydroxypinnatasterone (**177**), isolated from *Vitex cymosa*[125], (24*R*)-11α,20,24-trihydroxyecdysone (**178**)[126], and 11α,20,26-trihydroxyecdysone (**179**)[126] and 24-methylshidasterone (**180**)[127], isolated from *Vitex canescens*.

4.10. Liliaceae

The literature survey indicates that one new ecdysteroid, stachysterone A-20,22-acetonide (**181**), has been isolated from *Asparagus filicinus* Buch.-Ham. (Liliaceae)[128].

4.11. Limnanthaceae

The literature survey indicates that one new ecdysteroid, namely 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, lygodiumsteroside 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,22*R*)-3-[(β-D-glucopyranosyl)oxy]-2,14,20,22-tetrahydroxy-6-oxo-cholest-7-en-25-yl acetate} (**184**), pterosterone-3-*O*-β-D-glucopyranoside [(2β,3β,5β,22*R*,24*S*)-2,14,20,22,24-pentahydroxy-6-oxo-cholest-7-en-3-yl β-D-glucopyranoside] (**185**), ecdysone-3-*O*-β-D-glucopyranoside [(2β,3β,5β,22*R*)-2,14,22-trihydroxy-6-oxo-cholest-7-en-3-yl β-D-glucopyranoside] (**186**), isolated from the plant *Sida rhombifolia*[131], 20-hydroxy-24-hydroxymethyl ecdysone (**187**), isolated from the plant *Sida spinosa*[132], and glutinosterone [(20*R*,22*R*)-1β,2β,3β,14α,20,22,25-heptahydroxy-5β-cholest-7,24 dien-6-one] (**188**), isolated from the plant *Sida glutinosa*[133].

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 jollyanum*[134], cycleasterone A (**191**), isolated from *Cyclea barbata* Miers[135], 3-deoxy-1β,20-dihydroxyecdysone (**192**), 2-deoxy-5β,20-dihydroxyecdysone (**193**), and diploclidine (**194**) from *Diploclisia glaucescens*[136–138] and fibraurecdyside A (**195**), isolated from *Fibraurea tinctoria* Lour[139].

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 polypodine B 2-β-D-glucoside (**198**), isolated from the plant *P. vulgare*[140], 20-deoxymakisterone A (**199**), 25-epimer of amarasterone A (**200**), 25-deoxyecdysone 22-β-D-glucoside (**201**) from *Microsorum scolopendria*[141], *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-L-rhamnopyranoside (**204**), isolated from *Microsorum membranifolium*[142,143] and ponasteroside B (**205**), isolated from *Lepidogrammitis drymoglossoides*[144].

4.16. Polyporaceae

From the Polyporaceae family, the conducted literature survey indicates that five new ecdysteroids, (20*S*,20*R*,24*R*)-16,22-epoxy-3β,14α,23β,25-tetrahydroxyergost-7-en-6-one (**206**), (23*R*,24*R*,25*R*)-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 umbellatus*[145,146].

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 cuspidate*[148], ponasterone A 20,22-*p*-hydroxybenzylidene acetal (**215**), and ponasterone A 20,22-acetonide (**216**), isolated from the plant *Taxus canadensis* Marsh[149].

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, antidiabetic, hypolipidemic, and hepatoprotective effects[150–153]. 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 ajugacetalsterone E (**158**) exhibited *in vitro* cytotoxic activity, superoxide anion generation, and elastase release in *N*-formyl-methionyl-leucyl-phenylalanine/cytochalasin B (FMLP/CB)-induced human neutrophils[119]. 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 stress[43]. Another PE derivative cyasterone along with chikusetsusaponin IV could significantly inhibit inflammatory and oxidative enzyme levels, such as thromboxane A2 (TXA2), endothelin (ET), malondialdehyde

Table 1 Pharmacological effects of new phytoecdysteroids (since 1999) based on *in vitro* and *in vivo* studies.

Name of the ecdysteroids	Pharmacological activity	Study model	Cell line used	Conc./dose	Effect	Mechanism	Ref.
2,22-Dideoxy-20-hydroxyecdysone 25-*O*-β-D-glucopyranoside (**5**), 2,22-dideoxyecdysone 25-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**6**)	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	41
20,26-Dihydroxy 28-methyl ecdysone (**10**), 20,26-dihydroxy 24(28)-dehydro ecdysone (**11**), 20-hydroxyecdysone 22-glycolate (**12**)	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	43
3β,14α-Dihydroxy-5β-pregn-7-ene-2,6,20-trione (**14**)	Evaluated effects on germination and growth of *Lactuca sativa* L. and have shown little influence on germination as well as on shoot length.	*Ex situ*	*Lactuca sativa*	10−1000 μmol/L	Seed germination inhibition	Phytotoxicity	44
Niuxixinsterone D (**21**)	Evaluated inhibitory effects against LPS-induced NO production in RAW 264.7 macrophagesand exhibited anti-neuroinflammatory activity with inhibited 29.7 and 26.0% NO production.	*In vitro*	RAW 264.7 macrophages	50 μmol/L	Anti-neuroinflammatory activity	Inhibition of nitric oxide production	47
Aervecdysteroid A (20,25-epoxy-2β,3β,14α,22β-tetrahydroxy-5b-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**)	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	51
(20*R*)-22-Deoxy-20,21-dihydroxyecdysone (**36**)	Evaluated agonistic activity in the *Drosophila melanogaster* BII cell bioassay and shown good activity.	*In vitro*	*Drosophila melanogaster* B_{II} cell bioassay	ED_{50} 20 μmol/L	Agonistic activity	Growth inhibition	54
Septanoecdysone (**37**)	Evaluated antioxidant activity by using DPPH, $ABTS^{+}$, Fe^{3+} 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	55

(*continued on next page*)

Table 1 (*continued*)

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, anti-microbial	Free-radical-scavenging; growth inhibitory	68
3-*epi*-20-Hydroxyecdysone (**64**), ecdysone 22-acetate (**65**), (25*S*)-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* B_{II} 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**)	Evaluated toxicity in the oral aphid *Acyrthosiphonpisum* (Harris) test and exhibited low toxicity.	*In vitro*	(*Acyrthosiphonpisum* Harris)	LC_{50}> 100 ppm; LC_{50} = 48.5 ppm	Oral aphid test	Cytotoxic activity	76
(24*R*)-24-(2-Hydroxyethyl)-20-hydroxyecdysone (**87**)	Evaluated antioxidant activity on AAPH. -induced hemolysis of human RBC and Fe^{2+} + cysteine-induced lipid peroxidation of liver microsome and showed significant activity.	*In vitro*	*E. coli*; liver microsome	10 μL	Antioxidant activity; lipid peroxidation inhibition	Free-radical-scavenging; iron chelation; blood hemolysis prevention	79
3-*O*-*β*-D-Glucopyranosyl-3*β*,25-dihydroxy-5*β*-cholest-7-en-6-one-25-*O*-*β*-D-glucopyranoside (**119**)	Evaluated cytotoxic activity against two cancer cell lines of A549 (human lung adenocarcinoma) and HeLa cells (human epitheloidcervix carcinoma) by using the MTT assay and no significant activity have been observed.	*In vitro*	Lung cancer cell line (A549); leukemiacell line (HeLa)	12.5−100 μmol/L	Cytotoxic activity	Growth inhibitory	96
2,3-Diacetate-22-benzoate-20-hydroxyecdysone (**120**)	Evaluated cytotoxicity, antiproliferative activities in HeLa, HepG-2, and MCF-7 cell linesand 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
3*α*,14*α*,22*R*,25-Tetrahydroxy-5*β*(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

(20*R*)-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
(22*R*,24*R*,25*S*,26*S*)-2β,3β,14α,20*R*-Tetrahydroxy-26α-methoxy-6-oxo-stigmast-7-ene-22,26-lactone (**149**), (22*R*,24*R*,25*S*)-2β,3β,14α,20*R*,26*S*-pentahydroxy-6-oxo-stigmast-7-ene-22,26-lactone (**150**),(22*R*,25*S*)-2β,3β,14α,20*R*,24*S*-pentahydroxy-6,26-dioxo-stigmast-7-ene-22,26-lactone (**151**)	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. viridians* 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, GepG2, 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
21-Hydroxyshidasterone (**172**), 11β-hydroxy-20-deoxyshidasterone (**173**), 2,3-acetonide-24-hydroxyecdysone (**174**)	Evaluated anti-inflammatory activity on rat and significant activity have been observed.	*In vivo*	Carrageenan-induced inflammation in Sprague–Dawley Rats	100 mg/kg	Anti-inflammatory and analgesic activities	Inhibition of paw oedema inflammation, inhibition of histamine and serotonin	123
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	124

(*continued on next page*)

Table 1 (*continued*)

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 improvemen, 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	ID_{50} 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 and HeLa cell lines	MDA-MB-231, IC_{50} = 27.6, 21.6 and 15.8 μmol/L; HeLa IC_{50} = 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; EC_{50}, half maximal effective concentration; ID_{50}, median infectious dose; LC_{50}, 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)[154]. PE 22-*epi*-ajugasterone C (**62**) also showed *in vitro* antioxidant activity by DPPH radical scanging assay[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 *V. doniana* led to purification of three new PEs: (21-hydroxyshidasterone, 11β-hydroxy-20-deoxyshidasterone, and 2,3-acetonide-24-hydroxyecdysone) as well as several known PEs (ecdysteroids shidasterone, ajugasterone C, 24-hydroxyecdysone and 11β,24-hydroxyecdysone). The PEs 21-hydroxyshidasterone (**172**), 11β-hydroxy-20-deoxyshidasterone (**173**), and 2,3-acetonide-24-hydroxyecdysone (**174**) displayed significant inflammation-inhibitory effects on rat paw edema development induced by carrageenan in Sprague−Dawley rats[123]. PE niuxixinsterone D (**21**) was tested for inhibitory effects against lipopolyschharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophages. Niuxixinsterone D and serfurosterone A showed anti-neuroinflammatory activity by inhibiting NO production by 29.7% and 26.0%, respectively[47]. Another PE 3α,14α,22*R*,25-tetrahydroxy-5β(H)-cholest-7-en-6-one (**126**), isolated from *A. gypsophiloides*, was shown to possess anti-inflammatory and analgesic activities[100]. A recent report also showed various biological activities of PEs which were isolated from *Silene*genus. α-Ecdysone, in particular, was investigated for possible immunomodulatory and anti-inflammatory effects on LPS-treated RAW 264.7 macrophage cells and in a zebrafish model[155]. The compound showed potent immunostimulatory effects by enhancing membrane fluidity and lysosomal enzyme activity. α-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-κB) activity, as well as a reduction in mitogen-activated protein kinases (MAPKs) and protein kinase B (Akt) activation[155]. Some of the known PEs, such as paristerone, ecdysterone, and capitasterone isolated from the stems of *Diploclisia glaucescens*, have shown significant anti-inflammatory activity with half maximal inhibitory concentration (IC_{50}) values ranging from 1.5 to 11.6 μmol/L, respectively; this was discovered by measuring the inhibitory ratios of β-glucuronidase release in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factors[156]. 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[157]. 20-HE has been shown to improve dermal thickness[151,158] and promote wound-healing *in vivo*[13,150]. Additionally, 20-HE was also found to enhance keratinocyte differentiation[153], increase protein synthesis *via* Akt activation[159], and inhibit collagenase activity *in vitro*[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[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[161]. PEs also possess a promising future in skin care due to their perception as being a safe material, their abundance, and their sustainability[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[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[13], through a probable mechanism involving the activation of protein kinase B (also known as Akt)[105]. Calonysterone (**132**) has exerted a stronger effect on the Akt phosphorylation in mammalian skeletal muscle cells than the more abundant 20-HE[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 mytotube cell line C2C12[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[163]. 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 β-cells[165]. PEs extracted from the plant *Ajuga iva* (Lamiaceae) have exhibited *in vivo* potential therapeutic effects against experimental diabetes induced by alloxan[166]. The extracted PE caused reduction in blood glucose levels in the diabetic rats; it also reduced the levels of blood urea nitrogen, creatinine, triglycerides, 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

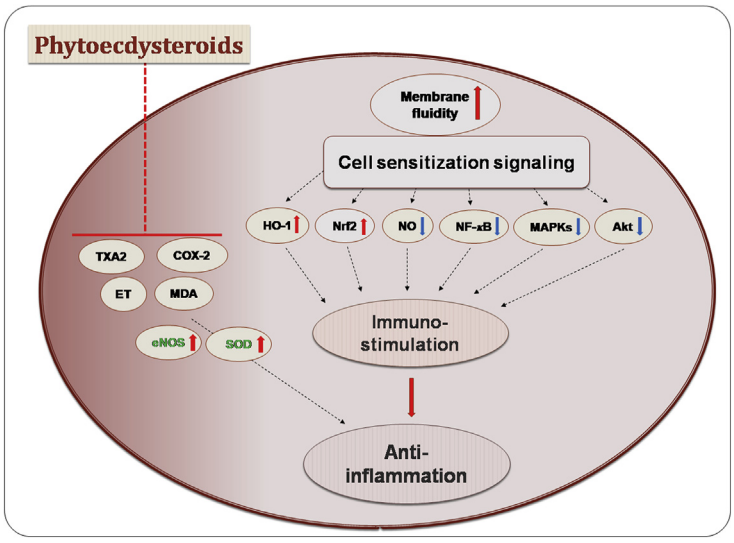

Figure 10 Overview of anti-inflammatory effects of phytoecdysteroids. Phytoecdysteroids tend to interfere with the membrane fluidity which leads to cell sensitization and modulate various cellular signaling processes in immunomodulation and inflammation. This especially involves the inhibition of NF-κB, COX-2, MDA, MAPKs and Akt, and activation of HO-1, Nrf2, eNOS and SOD. Akt, protein kinase B; COX-2, cyclo-oxygenase-2; eNOS, endothelial nitric oxide synthase; ET, endothelin; HO-1, hemaoxygenase-1; MAPKs, mitogen-activated protein kinases; MDA, malondialdehyde; NF-κB, nuclear factor-κB; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase; TXA2, thromboxane A2.

was also reported[166]. 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[167]. 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[168]. 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 (**62**), which has shown DPPH radical scanging activity followed by antimicrobial properties. It showed a weak scavenging effect as compared with myricetin and exhibited antimicrobial properties

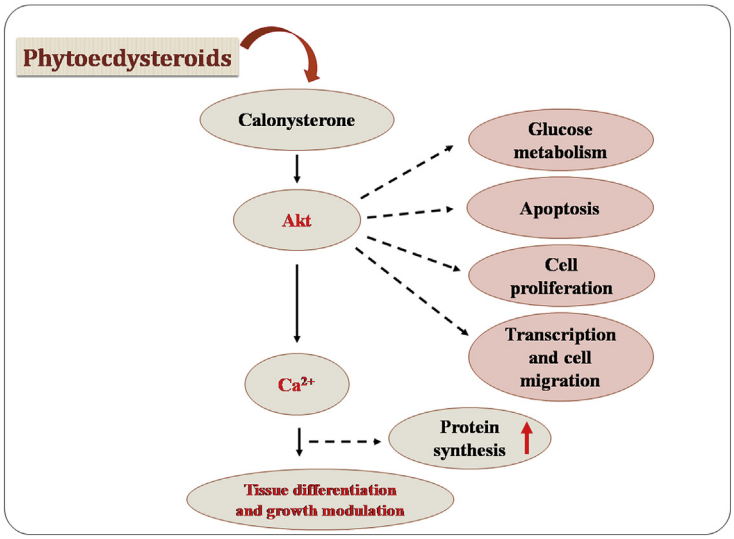

Figure 11 Tissue differentiation and growth modulatory activities of phytoecdysteroids. Calonysterone specifically alteres tissue differentiation and growth modulation, and affects cell proliferation and survival signalling mechanisms. Akt, protein kinase B.

against multi-resistant strains, such as *Staphylococcus aureus*, *Escherichia coli*, *Serratia* sp., *Klebsiella pneumoniae*, and *Candida albicans*[68]. (22*R*,24*R*,25*S*,26*S*)-2β,3β,14α,20*R*-Tetrahydroxy-26α-methoxy-6-oxo-stigmast-7-ene-22,26-lactone (**149**), (22*R*,24*R*,25*S*)-2β,3β,14α,20*R*,26*S*-pentahydroxy-6-oxo-stigmast-7-ene-22,26-lactone (**150**), and (22*R*,25*S*)-2β,3β,14α,20*R*,24*S*-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)-dehydroamarasterone B, isolated from the seeds of *L. carthamoides*, has show potent growth-inhibitory activity against *Drosophila melanogaster* B_{11} cells with median effective dose (ED_{50}) of 0.52 μmol/L[169]. Ajugacetalsterone E and four other neoclerodane diterpenoids from *A. decumbens* inhibited cell proliferation of the MCF-7, GepG2, A549, and B16 cell lines as an anticancer prospect[119]. 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 μmol/L, while selected iridoid glycosides exhibited more significant cytotoxicities with IC_{50} values ranging between 7 and 14 μmol/L[170]. A lipophilic *Leuzea* root extract containing the major PE 20-HE demonstrated potent cytotoxic effects on MCF-7 cells. The extract exhibitied cell proliferation with IC_{50} 30 μg/mL, yet 20-HE alone was not as effective[171]. Genome-wide expression analysis for differential transcriptional expression of 241 genes showed that *Leuzea* extract treatment was comparatively more effective that treatment with tamoxifen, a commonly used medication for preventing breast cancer in women[171]. The cytotoxic potentials of *n*-butanolic fraction of *Ajuga chamaecistus*, 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 μg/mL concentration of the extract[172]. Additionally, a previous study using three major compounds (20-HE, cyasterone, and 8-acetylharpagide) 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[173]. 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 MTT 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[174]. 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[175]. 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 ecdysterone 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, ecdysone, was isolated from the silkworm *Bombyx mori*[2]. In *Spinacia oleracea* L. (spinach), the major ecdysteroid is ecdysterone, which can occur in concentrations between 50 and 800 μg 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 ecdy receptor 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[13,157]. 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[178]. The rate of free-radical formation was reduced by 20-HE in cholesterol-dependent manner, while α-tocopherol showed lower antioxidant

effect in the membrane. 20-HE showed potent antioxidant action in combination with α-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 griping 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 (GADD45α) and tumor protein p53 inducible nuclear protein 1 (TP53INP1) pointed to cell cycle arrest at the G1/S-transition checkpoint in human bread 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 intereconnective 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

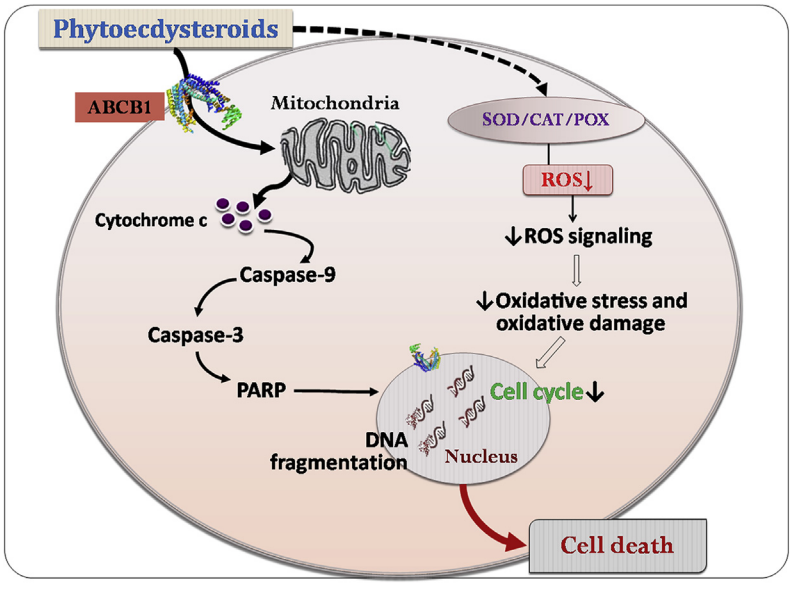

Figure 12 Proposed mechanism of the anticancer effects of phytoecdysteroids through induction of cell death. Phytoecdysteroids interact through the ABCB1 transmembrane protein and activates mitochondrial apoptosis pathway as well as it suppresses oxidative stress and causes cell cycle arrest. These collectively 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.

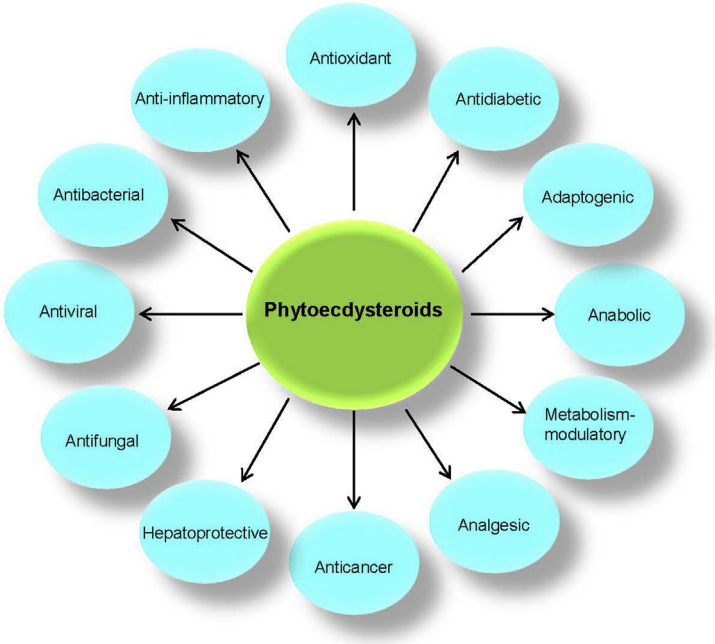

Figure 13 Summary of various biological and pharmacological effects of ecdysteroids.

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 Eunüs 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

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2020.10.012.

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