PLANT POLYACETYLENIC GLYCOSIDES: OCCURRENCE, BIOSYNTHESIS AND BIOLOGICAL ACTIVITIES

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Summary

The aim of the present review is to focus a small, relatively new group of natural products derived from two plant families, the polyacetylene glycosides (PAGs) and their biological activities. PAGs are glycosylated derivatives of polyacetylenes which possess many useful properties such as, antioxidant, antidiabetic, immunomodulatory (T-cell modulator), antihistaminic, anti-inflammatory and cytotoxic to tumor cells. However, so far only few PAGs could be isolated from members of the family Asteraceae and Campanulaceae, particularly from *Bidens* species. These plants are common in Chinese and Japanese traditional medicines. PAGs have been isolated from different parts (leaves, flowers, roots and rhizomes) of these plants and from callus and hairy root cultures. Despite the fact that these PAGs possess many useful biological activities their potential has not yet been investigated sufficiently thoroughly, and which have not been exploited by the pharmaceutical industry. Most certainly, their biosynthesis in plants still very poorly understood compare to the biosynthesis of ploacetylenes. Here, for the first time we gathered and compiled the information regarding to the occurrence, biosynthesis and biological activities of PAGs. This information certainly is helpful for those working on plant PAGs and further generates interest in others as well.

Key Words: Antiallergic; Antidiabetic; Asteraceae; Campanulaceae, Polyacetylenic glycosides (PAGs); Polyacetylene

Introduction

Polyacetylenic glycosides (PAGs), reported from few members of family Asteraceae and Campanulaceae represents one of the class of bioactive plant secondary products among others (1), (2), (3), (4), (5), (6) (7). So far 22 PAGs are known, however most of them have been isolated from *Bidens* species (1), (2), (4), (5), (6) (7). PAGs are glycosides of polyacetylenes in which a sugar (glucose or rhamnose) moiety is joined to polyacetylene through –O- glycosidic linkage. The occurrence, biosynthesis, and biological activities of polyacetylenes in family Apiaceae has been extensively reviewed by Christensen and Brandt, 2006 (8). Polyacetylenes functions as bioactive secondary metabolites, but considered undesirable in plant foods due to their toxicant properties (8). Perhaps, polyacetylenes possess many biological properties such as anti-angiogenic and cytotoxic activities (9), (10), (11). Likely, PAGs also demonstrated useful biological activities *viz.,* antidiabetic, T-helper cell modulator, antihistaminic and NO inhibitor (1) (4) (5) (6) (7). Though, PAGs could be promising molecules in medicines and pharmaceuticals due to their specific biological activities, they have not been studied extensively. In the present review we bring in to line the information about their occurrence, biosynthesis and biological activities in view of their possible applications in medicines and pharmaceuticals. This information may provide deeper insight in to PAGs so the maximum health benefit can be harnessed from these plant secondary products.

Occurrence and functions

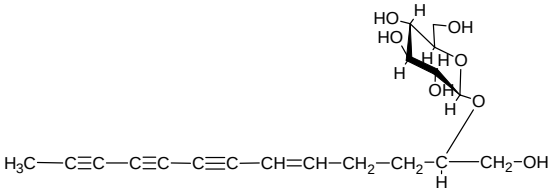
Most of the PAGs known so far have been isolated from few plants belongs to family Asteraceae (*Bidens pilosa B. parviflora* Wild, *B. bipinnata* Linne, *Gymnaster koraiensis, Carthamus tinctorius, Atractylodes ovata, A. Lancea, A. yunnanensis*) and Campanulaceae (*Pratia nummularia, Lobelia cardinalis*). These plants are commonly used in Chinese and Japanese traditional medicines.

However, most of the PAGs were isolated from *Bidens* species (1), (2), (4), (5), (6) (7). The functions of PAGs in plants, however yet not fully understood. Table 1 summarizes the information on occurrence of PAGs in plants while their structures are depicted in Figure 1.

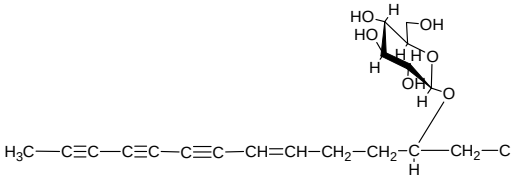
Table 1: Occurrence of polyacetylenic glycosides (PAGs) in plants

Family	Plant	Compound	Plant parts	References
Asteraceae	*Bidens pilosa*	**1, 2, 3**	Aerial parts	Ubillas et al., 2000; Chang et al., 2004; Chiang et al., 2007
	B. parviflora Wild	**4, 5, 6, 7, 8**	Whole plant	Wang et al., 2001
	Gymnaster koraiensis	**9, 17**	Roots	Park et al., 2002
	Carthamus tinctorius	**10, 11, 12**	Flowers	Zhou et al., 2006
	B. bipinnata (Linne)	**13,14**	Aerial parts	Li et al., 2004
	Atractylodes ovata *A. lancea*	**16, 20**	Rhizome	Kitajima et al., 2003a,b; Japanese Pharmacopoeia; 2001
	Aster yunnanensis	**21**	Roots	Shao et al., 1995
Campanulaceae	*Pratia nummularia;* *Lobelia cardinalis*	**15,18,19, 22**	Callus and hairy root	Ishimaru et al., 2003; Yamanak et al., 1996

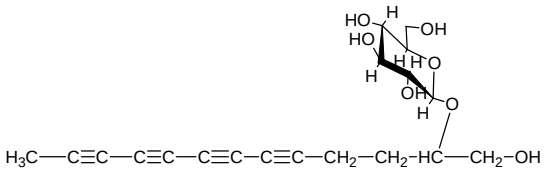
Figure 1: Structures of plant polyacetylenic glycosides (PAGs)



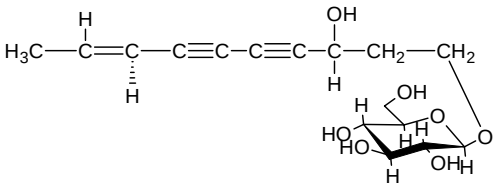
2-β-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7, 9, 11-triyne **(1)**



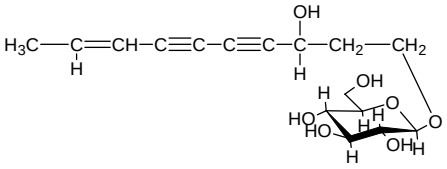
3- β-D-glucopyranosyloxy-1-hydroxy-6(E)-tetradecene-8,10,12-triyne **(2)**



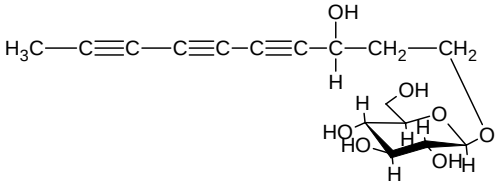
2- β-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne or Cytopiloyne **(3)**



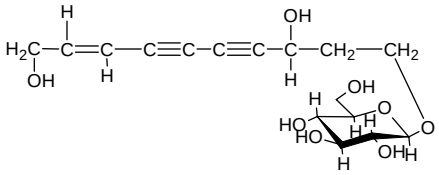
Bidensyneosides A1 **(4)**



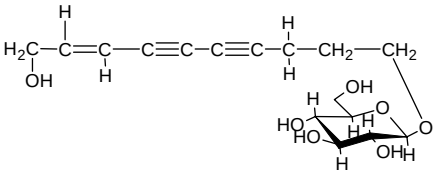
Bidensyneosides A2 **(5)**



Bidensyneosides B **(6)**

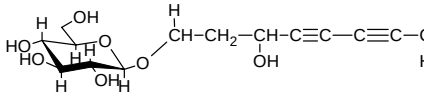


Bidensyneosides C **(7)**

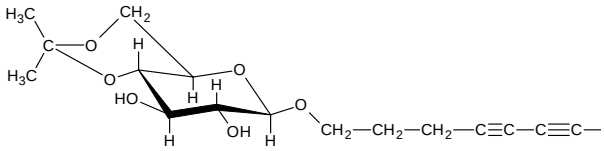


3-deoxy-bidensyneosides B **(8)**

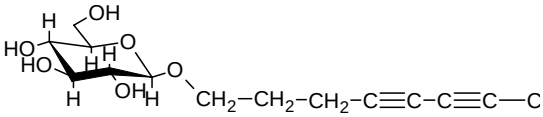
Figure 1: Cont…



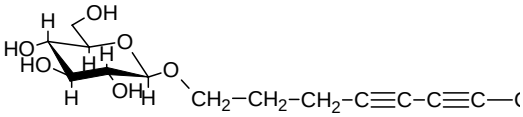
Gymnasterkoreasides A **(9)**



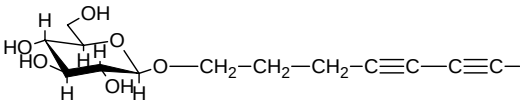
Carthamoside A1 **(10)**



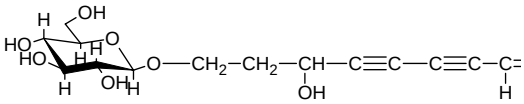
Carthamoside A2 **(11)**



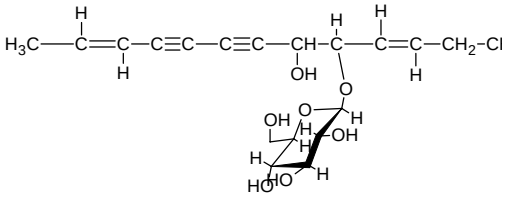
8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside **(12)**



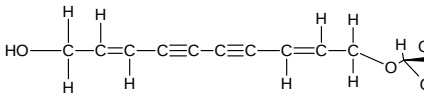
Bidenoside C **(13)**



Bidenoside D **(14)**

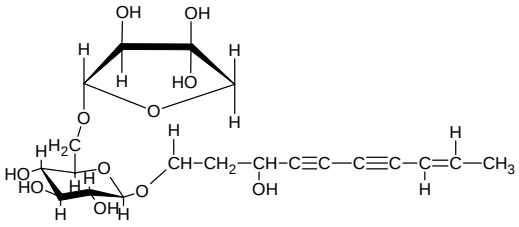


Lobetyolin **(15)**

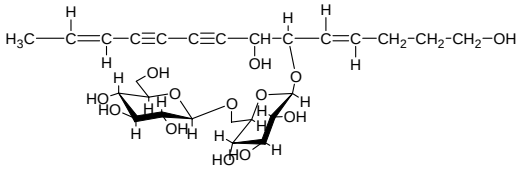


(2E,8E)-2,8-decadiene-4,6-diyne-1,10-diol 1-O- β-D-glucopyranoside **(16)**

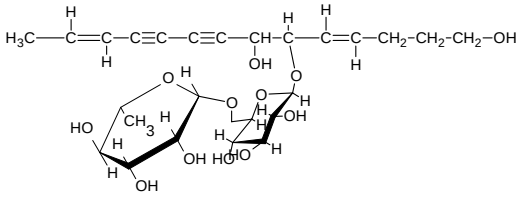
Figure 1: Cont…



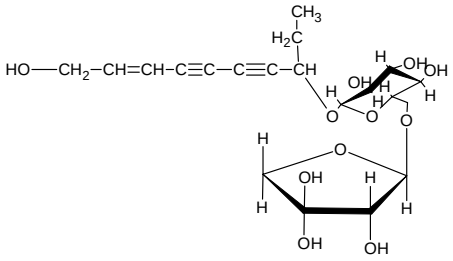
Gymnasterkoreasides B **(17)**



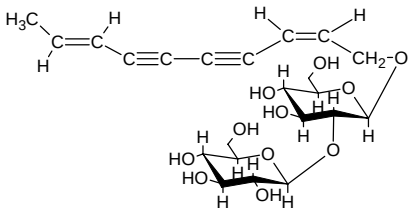
Lobetyolinin **(18)**



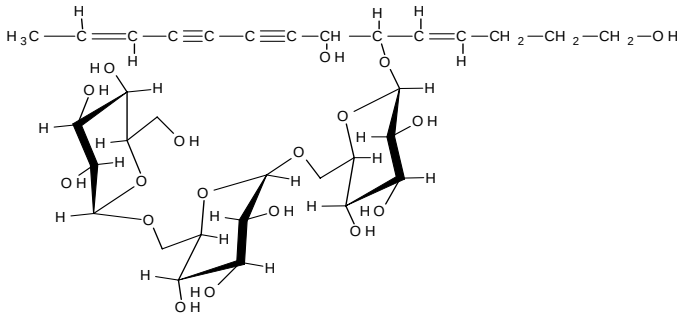
Pratialin A **(19)**



(2*E*)-2-decene-4,6-diyne-1,8-diol 8-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside **(20)**



Asteryunnanoside I **(21)**



Pratialin B **(22)**

Structural analysis

Chemically, PAGs are composed of polyacetylenes (linear chain of 10 or 14 carbon atoms with two or more triple bonds) and a sugar (glucose or rhamnose) moiety joined together by –O- glycosidic bond. Previously, PAGs structures have been extensively analyzed by the $^{1}H–^{1}H$ shift correlation spectroscopy (COSY), HMQC, and hetero-nuclear multiple-bond correlations (HMBC) spectra (12), (6), (7). These spectroscopic analyses have revealed the presence of two or three triple bonds in the polyacetylene part of various PAGs. The triple bonds were present at C4, C6 and C7, C9, C11 or C8, C10, C12 or C4, C6, C8 respectively in the polyacetylene chain. Cytopiloyne **(3)** a polyacetylene glycoside of *B. pilosa*, however had 4 triple bonds at C5, C7, C9 and C11 (4). The sugar moiety in PAGs has been identified by thin-layer chromatography (TLC) after hydrolysis of PAGs with β–glucosidase and from ^{1}H- and ^{13}CNMR spectral data (7). In general, sugar residue in most of the PAGs is linked to the terminal-C of polyacetylene. However, in some of the PAGs it is linked either to C2, C3 or C6 of polyacetylenes. Thus, the PAGs could be classified based on the size of polyacetylene chain, number and type of sugar residue and according to number of triple bonds in polyacetylenes. Based on the size of polyacetylene chain, PAGs are classified as C-10 and C-14 (Table 2) while according to the number of sugar residues present in PAGs they are classified as mono- , [examples 2-β-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7, 9, 11-triyne **(1),** 3- β-D-glucopyranosyloxy-1-hydroxy-6(E)-tetradecene-8,10,12-triyne **(2),** 2- β-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne or cytopiloyne **(3),** bidensyneosides A1 **(4),** bidensyneosides A2 **(5),** bidensyneosides B **(6),** bidensyneosides C **(7),** 3-deoxy-bidensyneosides B **(8),** gymnasterkoreasides A **(9),** carthamoside A1 **(10)**, carthamosode A2 **(11),** 8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside **(12),** bidenoside C **(13),** bidenoside D **(14),** lobetyolin **(15),** (2E,8E)-2,8-decadiene-4,6-diyne-1,10-diol 1-O- β-D-glucopyranoside **(16);** Di-[examples gymnasterkoreasides B **(17)**, lobetyolinn **(18),** pratialin A **(19),** (2*E*)-2-decene-4,6-diyne-1,8-diol 8-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside

(20) and asteryunnanoside I **(21)** and Tri-glucosides of polyacetylenes example pratialin B **(22)** (Table 2)**.** Only Pratialin-A, rutinoside of lobetyol represents the first example of a polyacetylene derivative having a rhamnose rather glucose in its structure (12). When classified based on number of triple bonds in polyacetylene, 18 out of 22 PAGs had two triple bonds, 3 had three triple bonds and only had four triple bonds (Table 2). The structures of various PAGs are depicted in Figure 1. The individual PAGs have been assigned trivial names besides their systematic names. The trivial names ending in either 'sides' such as bidensyneosides or 'lin' for example pratialin generally denote glycosides those extracted from the *Bidens* and *Pratia* species respectively.

Table 2: Classification of plant polyacetyelenic glycosides (PAGs) according to the size of the polyacetylene chain, nomuber of triple bonds and glucose residues.

PAGs	No. of C atoms	No. of triple bonds	No. of glucose
(2E,8E)-2,8-decadiene-4,6-diyne-1,10-diol 1-O-beta-D-glucopyranoside **[16]**	10	02 (C4 ; C6)	01 (C1)
8*Z*-decaene-4,6-diyn-1-*O*-b –D-glucopyranoside (bidenoside C) **[13]**	10	02 (C4 ; C6)	01 (C1)
8*E*-decaene-4,6-diyn-3,10-dihydroxy-1-*O*-b –D-glucopyranoside (Bidenoside D) **[14]**	10	02 (C4 ; C6)	01 (C1)
3(R), 8(E)-8-decene-4, 6-diyne-1, 3-diol 1-O-β-D-glucopyranoside (Bidensyneosides A1) **[4]**	10	02 (C4 ; C6)	01 (C1)
deca-3(R), 8(Z) 8-decene-4, 6-diyne-1, 3-diol 1-O-β-D-glucopyranoside (Bidensyneosides A2) **[5]**	10	02 (C4 ; C6)	01 (C1)
3(R)-deca-4, 6, 8-triyne-1, 3-diol 1-O-β-D-glucopyranoside (Bidensyneosides B) **[6]**	10	03 (C4; C6; C8)	01 (C1)
3(R), 8(E)-8-decene-4, 6-diyne-1, 3, 10-triol 1-O-β-D-glucopyranoside (Bidensyneosides C) **[7]**	10	02 (C4 ; C6)	01 (C1)
8(E)-8-decene-4, 6-diyne-1, 10-diol 1-O-β-D-glucopyranoside (3-deoxybidensyneoside B) **[8]**	10	02 (C4 ; C6)	01 (C1)

2beta-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne (Cytopiloyne) **[3]**	10	04 (C5;C7;C9;C11)	01 (C1)
4,6-acetonide-8*Z*-decaene-4,6-diyne-1-*O*-b –D-glucopyranoside (Carthamoside A1) **[10]**	10	02 (C4 ; C6)	01 (C1)

Table 2 Cont…: Classification of plant polyacetyelenic glycosides (PAGs) according to the size of the polyacetylene chain, nomuber of triple bonds and glucose residues.

PAGs	No. of C atoms	No. of triple bonds	No. of glucose
4,6-decadiyne-1-*O*-b -D-glucopyranoside (Carthamoside A2) **[11]**	10	02 (C4 ; C6)	01 (C1)
8*Z*-decaene-4,6-diyne-1-*O*-b -D-glucopyranoside **[12]**	10	02 (C4 ; C6)	01 (C1)
(3R)-8-decene-4,6-diyne-1,3-diol 1-O-beta-D-glucopyraside (Gymnasterkoreasides A) **[9]**	10	02 (C4 ; C6)	01 (C1)
(3R)-8-decene-4,6-diyne-1,3-diol 1-O-beta-D-apiofuranosyl-(1-->6)-beta-D-glucopyraside (Gymnasterkoreasides B) **[17]**	10	02 (C4 ; C6)	02 (C1)
2-β-D-glucopyranosyloxy-1-hydroxy-5(E)-tridecene-7,9,11-triyne **[1]**	14	03 (C8;C10;C12)	01 (C3)
3- β-D-glucopyranosyloxy-1-hydroxy-6(E)-tetradecene-8,10,12-triyne **[2]**	14	03 (C7;C9;C11)	01 (C2)
2Z,8E-decadiene-4,6-diyne-1-O-beta-D-glucopyranos yl-(1-->2)-beta-D- glucopyranoside (Asteryunnanoside I) **[21]**	10	02 (C4;C6)	02 (C1)
(2*E*)-2-decene-4,6-diyne-1,8-diol 8-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside **[20]**	10	02 (C4; C6)	02 (C1)
Lobetyolin **[15]**	14	02 (C8 ; C10)	02 (C6)
Lobetyolinin **[18]**	14	02 (C8 ; C10)	02 (C6)
lobetyol 9-O-glc(6)-(1)rha (Pratialin-A) **[19]**	14	02 (C8 ; C10)	02 (C6)
lobetyol 9-O-glc(6)-(1)glc(6)-(1)glc (Pratialin-B) **[22]**	14	02 (C8 ; C10)	03 (C6)

Biosynthesis

The biosynthesis of PAGs can be conveniently treated in two phases (Figure 2). The first phase involves biosynthesis of polyacetylene tail from unsaturated fatty acids by dehydrogenation (14), (15). Earlier studies on biosynthesis of falcarinol has revealed that polyacetylene of falcarinol is biosynthesized from oleic acid (unsaturated fatty acid) by dehydrogenation leading to the C18-acetylenes crepenynic acid and dehydrocrepenynic acid (14), (15). Likely, results of feeding experiments with ^{14}C labeled precursors have confirmed that polyacetylenes are built up from acetate and malonate units (14), (15), (16), (17), (18), (19), (20).

In the second phase, polyacetylenes thus formed get glycosylated at carbon 1, 2 and 6 by glucose. It is presumed that glycosylation of polyacetylenes is catalyzed by glucosyltransferases (Figure 2). Glycosylation reactions are very common in plants and mostly catalyzed by UDP-glucosyltransferases family of proteins. See the article by Jones and Vogt, 2001 (21) for roles of UDP-glucosyltransferases in the biosynthesis of plant secondary products. The extent of chemical diversity in PAGs is relatively limited compared to those found in other classes of plant secondary metabolites. However, the glycosylation of polyacetylenes at different carbon atoms seems to be responsible for formation of individual distinguished PAGs. The less amount of structural diversity, however, could be due to glycosylation of polyacetylenes which is restricted to C1, C2 and or C6 positions.

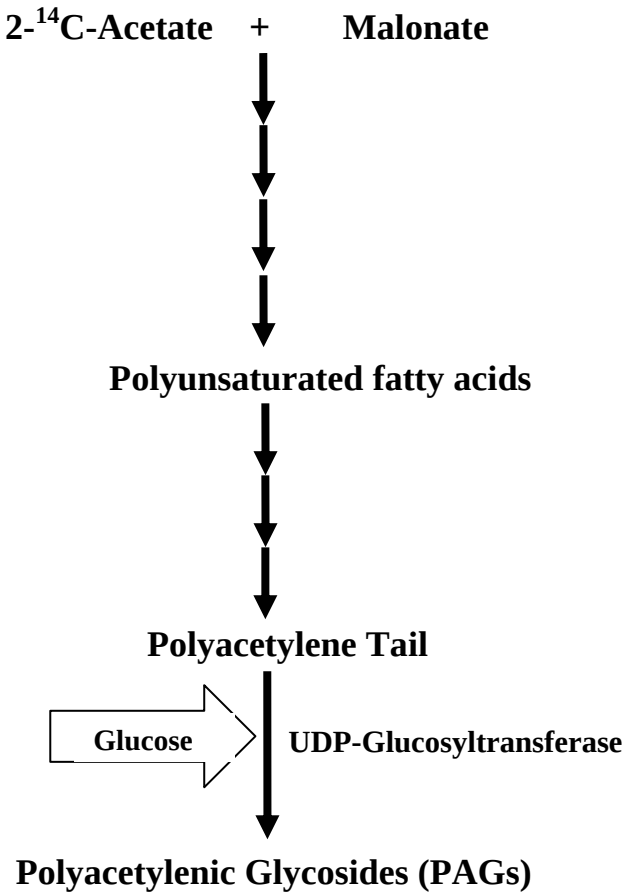


Figure 2: Plausible biochemical pathway of Polyacetylenic glycoside biosynthesis in plants

Biological significance

The PAGs could be of great significance in medicines and pharmaceuticals due to their broad spectrum biological activities. The PAGs have shown their potential as antidiabetic, T-helper cell modulator, antihistaminic and NO inhibitor (1) (4) (5) (6) (7). However, these plant products have yet to be clinically tested. Here, we describe some of the important biological activities of PAGs. The summary information of biological activities of PAGs is presented in Table 1.

Antidiabetic activity/ T-helper cell modulator

Most elaborated work on antidiabetic and T-helper cell modulating activity has been carried on PAGs those isolated from *Bidens* species (4), (5), (6). *Bidens pilosa* (Linn.) belongs to the family Asteraceae and commonly used in traditional medicine as an antiinflammatory, diuretic, anti-rheumatic, antibiotic, or antidiabetes folk medicines (22). The genus *Bidens* has been abundantly studied for their phytochemical compositions and biological activities and polyacetylenes was reported as one of the major chemical constituents (1) (2) (7). Previously, PAGs with potential anti-diabetic activity in NOD (Non Obese Diabetes) mice (6), *db*/*db* mice (1) and alloxan-treated mice (23) have been isolated from aerial parts of *Bidens pilosa* (Linn). These authors have evaluated anti-diabetic potential of the PAGs by bioactivity-directed strategy using T cell differentiation assays. *Bidens pilosa* contained two PAGs, 2-β-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7, 9, 11-triyne **(1)** and 3- β-D-glucopyranosyloxy-1-hydroxy-6(E)-tetradecene-8,10,12-triyne **(2)** that prevents the onset of diabetes in NOD mice and *db*/*db* mice (1), (6). Very recently, Chiang et al., 2007 (4) have isolated a novel bioactive PAG, 2-β-D-glucopyranosyloxy-1-hydroxy-trideca-5, 7, 9, 11-tetrayne also referred as cytopiloyne **(3)** from the *Bidens pilosa***.** Like other PAGs, cytopiloyne **(3)** also inhibits Th1 cell differentiation but increased Th2 cell differentiation in mouse T cells. Unlikely, to the antidiabetic potential assay, the

effect of cytopiloyne on modulating T cell differentiation was evaluated using $CD4^+$ T cells isolated from lymph nodes of BALB/c mice. The results of effect of cytopiloyne on T cell differentiation has revealed that cytopiloyne decreases the percentage of INF-γ producing cells (i.e. Th1 cells) significantly from 72.0% to 59.8% and addition of cytopiloyne to the differentiating Th cells increased the percentage of mouse IL-4-producing cells (i.e. Th2 cells) considerably (4). Also, cytopiloyne **(3)** suppressed IFN-γ expression and promoted IL-4 expression in mouse splenocytes *ex vivo*. The PAGs of *Bidens* spp. thus can be used to treat T cell-mediated immune diseases such as diabetes.

Antiallergic activity

The PAGs have the ability to inhibit NO (nitric oxide) and histamine release from rat mast cells. Such NO inhibitory PAGs namely bidensyneosides A1, A2, B, C and 3-deoxybidensyneoside B (**4, 5, 6, 7, 8**) have been isolated from *Bidens parviflora* wild (7). *B. parviflora* (wild) is used in traditional Chinese medicine as an antipyretic, anti-inflammatory, and antirheumatic (24), (25). The PAGs of *B. parviflora* inhibits NO production in lipo-polysaccharide and interferon-λ activated murine macrophages (RAW264.7) and histamine release from rat mast cells (7). The antihistamine release activities of these PAGs were evaluated on rat peritoneal exudate cells induced by the antigen–antibody reaction.

Cytotoxic activity

Park et al., 2002 (12) have reported two PAGs gymnasterkoreasides A and B (**9, 17**) in BuOH-soluble fraction of the roots of *Gymnaster koraiensis* (Nakai) Kitamura. Earlier, eight polyacetylenes were isolated from the roots of *G. koraiensis* with cytotoxic activity against L1210 tumor cells (26). However, cytotoxic activity of gymnasterkoreasides A and B has yet not studied.

Other biological activities

Few more plants of Chinese and Japanese traditional medicine system medicinal systems have been investigated for the PAGs. Three PAGs, carthamoside A1, A2 and 8*Z*-decaene-4,6-diyne-1-*O*-β-D-glucopyranoside (**10, 11, 12**) were isolated in air dried flower extract of one such plant *Carthamus tinctorius* L. (3). The plant is reported to promote blood circulation by removing blood stasis. Similarly, *Bidens bipinnata* Linne the plant used in folk medicine against various diseases such as inflammation, rheumatism, sore throat, hypertension and diabetes type I possess two polyacetylenic glucosides bidenoside C and D (**13, 14**) (2)**.** Li et al. 2004 (2) have also isolated four PAGs, lobetyolin, lobetyolinin, pratialin-A and pratialin-B **(15, 18, 19, 22)** from callus and hairy root cultures of *Pratia nummularia* (Lam.) the plant used traditionally for the treatment of pus, contusion, cough and an anti-inflammatory. *Atractylodes lancea* and *A. ovata* yet are another plants contains PAGs (2*E*, 8*E*)-2, 8-decadiene-4, 6-diyne-1, 10-diol 1-*O*-β-D-glucopyranoside **(16)** and (2*E*)-2-decene-4,6-diyne-1,8-diol 8-*O*- β-D-apiofuranosyl-(1-6)- β-D-glucopyranoside (**20**) respectively (27) (28). The rhizomes of *Atractylodes* plants have been used as an important crude drug since antiquity. They are listed in the Chinese, Korean, and Japanese Pharmacopoeias and are prescribed in traditional medicine as diuretic and stomachic drugs (29). Asteryunnanoside I (**21**) isolated from the roots of *Aster yunnanensis* aids to the number of plant PAGs (30). Though, these PAGs have not been tested for their biological activities in animal models, however, broadly the PAGs found in these medicinal plants could be partially attributed for their respective biological activities.

Determination

So far, no qualitative and quantitative chromatographic methods have been described for the determination of PAGs in plant samples. However, several chromatographic methods are available for the qualitative and quantitative determination of polyacetylenes in plant samples such as, HPLC combined with

UV-detection, capillary gas chromatographic techniques (GC–FID and/or GC–MS) and liquid chromatography combined with mass spectrometry (LC–MS/MS). See review by Christensen and Brandt, 2006 (8) and references there in for the chromatographic methods of polyacetylene determination in plants. Likely, the polyacetylenes in the PAGs could also be determined after removal of sugar (glycone) by hydrolyzing PAGs with sulfuric acid. While the Based on this observation the PAGs can be determined indirectly by determining the polyacetylene and glucose separately. The glucose (glycone) can be determined after derivitizing with trimethylsilyl (TMS) thiazolidine derivative by gas-liquid chromatography (GLC) (12).

Conclusion

Although, plant PAGs are small and relatively less studied small group of new plant secondary metabolites, their biological potential particularly as antidiabetic, T-helper cell modulator, antiallergic and antihistaminic seems to be promising for medicinal and pharmaceutical use. Therefore, much attention is needed for harnessing maximum benefit from plant PAGs so they could be transformed in to lead or novel pharmaceuticals or therapeutics. In the present review we covered the occurrence and biological activities of PAGs and hypothesize a plausible route of their biosynthesis in plants. However, we also tried to focus some major shortfalls which may have severe impediment on growth and development PGAs researches (1) At present, our knowledge about PAGs seems to be very limited to their isolation and biological activities in the experimental animals. (2) Another key question is of their preclinical and clinical trials *in vivo* to ensure their potential as lead pharmaceuticals and (3) No chromatographic method has been developed so far for the determination and evaluation of their bioactive potential. These shortcomings are the challenges for the researchers working on plant PAGs. Hence, these are exciting times for searching more number of valuable PAGs from other plant families, microbes and marine organisms as well.

Successful preclinical and clinical trials *in vivo* should be performed for development of therapeutic PAGs. Apart from these, equal efforts should be made to understand the biosynthesis and regulation of PAGs in plants which will results in isolation and identification of the genes and enzymes involved in their biosynthesis. The present review will provide infant information that certainly stimulates the programs and efforts aimed at PAGs.

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