New advances in chemical defenses of plants: researches in calceolariaceae

ARTICLE *in* PHYTOCHEMISTRY REVIEWS · JANUARY 2015

Impact Factor: 2.41 · DOI: 10.1007/s11101-014-9392-y

Phytochem Rev
DOI 10.1007/s11101-014-9392-y

New advances in chemical defenses of plants: researches in calceolariaceae

Carlos L. Cespedes · Pedro M. Aqueveque · José G. Avila · Julio Alarcon · Isao Kubo

Received: 20 September 2014 / Accepted: 29 December 2014

Abstract Evidences about the biosynthesis of secondary metabolites from plants point to constitutive or induce chemical defense generated for protection against to different phytopathogenic attack. *Calceolaria* spp. is regarded both as a notorious weed and as a popular ornamental garden plant and have medicinal application. Some taxa of the America distributed *Calceolaria* genus are toxic to insects, fungi and several bacteria strains, and its effect has been associated with the presence of phenolics. *Calceolaria* spp. produces a number of iridoids, flavonoids, naphthoquinones and phenylpropanoids that have been shown to possess interesting biological activities. All these aspects are considered in this review to allow an evaluation of the potential for utilization of the large biodiversity of *Calceolaria* available. An up-to-date of the phytochemistry and biological activities of several members of the Calceolariaceae family is show. New iridoids, flavonoids and phenylpropanoids for these *Calceolaria* species have been isolated, identified and tested for their antifeedant, igr, insecticidal, antimicrobial, anticancer, proteinase, tyrosinase, and acetylcholinesterase inhibitory activities. Until now mixtures of flavonoids have been found to be potent insecticides and fungicides, followed by

Phytochemistry reviews. Por invitación (Prof. Jianbo Xiao).

C. L. Cespedes (✉)
Laboratorio de Bioquimica Vegetal y Fitoquimica-Ecologica, Grupo de Investigación: Química y Biotecnología de Productos Naturales Bioactivos, Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bio Bio, Av. Andrés Bello s/n, C.P. 3780000 Chillan, Ñuble, Chile
e-mail: Cespedes.leonardo@gmail.com

P. M. Aqueveque
Laboratorio de Micología Aplicada, Departamento de Agroindustrias, Facultad de Ingeniería Agrícola, Universidad de Concepción, Chillan, Chile
e-mail: pedroaqueveque@udec.cl

J. G. Avila
Laboratorio de Fitoquímica, UBIPRO, FES-Iztacala UNAM, Av. de los Barrios # 1, Los Reyes Iztacala., Tlalnepantla CP 54090, Estado de México, Mexico
e-mail: tuncomaclovio2000@yahoo.com

J. Alarcon
Laboratorio de Sintesis y Biotransformación de Productos Naturales, Grupo de Investigación: Química y Biotecnología de Productos Naturales Bioactivos. Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bio Bio, Chillan, Chile
e-mail: jualarcon@ubiobio.cl

I. Kubo
Chemical Ecology Lab., ESPM Department, University of California at Berkeley, Berkeley, CA, USA
e-mail: ikubo@berkeley.edu

Published online: 03 January 2015

phenylpropanoids mixtures and iridoids showed to be antifeedant and in some cases repellent and attractant. Dose-dependent experiments shows that flavonoids are insecticidal against *S. frugiperda* and *D. melanogaster* at early growth stages. Bactericidal and fungicidal activity showed that dunnione (a naphthoquinone) have potent activity as fungistatic and fungicidal. O-methylflavonols, and different mixtures of them were very effective fungistatic. However, fungistatic quercetin and dunnione both combined with sublethal amount of kaempferol and gallic acid showed a strong fungicidal activity against phytopathogenic strains. Additionally, naphthoquinones possess a promissory activity as anticancer.

Keywords Biological activity · Calceolariaceae · Naphthoquinones · Insecticidal · Antimicrobial · Anticancer

Introduction

Plants produce a great variety of secondary metabolites that do not have apparent function in physiological or biochemical processes; these compounds (or allelochemicals) are important in mediating interactions between plants and their biotic environment (Berenbaum 1989, 1990, 2002; Bowers 1981; Kessler and Baldwin 2002). Some of them can be used as leader molecules for the development of protective agents against insects and fungi (Cespedes et al. 2000, Céspedes et al. 2001a, b; Crombie 1999; Heath et al. 2014; Kubo 1997 Kubo et al. 1981, 1993, 2000, 2003a, b), and enzyme inhibitors (Calderón et al. 2001; Céspedes et al. 2001a, b, Cespedes et al. 2013a, b; Kubo et al. 2000, 2003a, b; Kubo 1997; Keane and Ryan 1999; Ortego et al. 1999). The increasing interest in the possible application of secondary metabolites for pest management has directed the investigation towards the search of new sources of biologically active natural products, with new modes, sites, and mechanisms of action (Gonzalez-Coloma et al. 1997; Conner et al. 2000; Eisner et al. 2000; Meinwald 2001). These characteristics may enhance their value as commercial pesticides (Akhtar et al. 2008, 2012; Akhtar and Isman 2013; González and Estevez-Braun 1998; Isman 2006; Isman and Akthar 2007; Isman and Grieneisen 2014; Miresmailli and Isman 2014; Valladares et al. 1997).

Here, we review new results about the bioactivity of extracts, fractions, mixtures and pure compounds from selected members of Calceolariaceae. Plants from this genus *Calceolaria* (Calceolariaceae: Scrophulariceae) are distributed in temperate and tropical regions in New Zealand and Central and South America (Engler 1964; Di Fabio et al. 1995; Garbarino et al. 2004). In America *Calceolaria*, with approximately 250 species ranges from Mexico to Tierra del Fuego, occurs mainly at high altitudes along the Andes but reaches sea level on both the Atlantic and Pacific coasts in Patagonia (Cosacov et al. 2009). Several species of *Calceolaria* are used as ornamental plants and in traditional medicine (Falcão et al. 2006). The aerial parts of these plants are used in Chile and South America due to their analgesic, digestive and diuretic properties (Sachetti et al. 1999) and as antimicrobial in stomach treatment (Sachetti et al. 1999; Garbarino et al. 2004). Some species of this genus have substances with potential use as: insecticide (Khambay and Jewess 2000), against tuberculosis (Woldemichael et al. 2003) and as growth inhibitors of TA3 tumour cells and methotrexate resistant TA3 cells (Morello et al. 1995). Flavonoids, glucophenylpropanoids, and diterpenes were identified in *Calceolaria* (Cespedes et al. 2014; Di Fabio et al. 1995; Garbarino et al. 2000; Muñoz et al. 2013a, b, c; Nicoletti et al. 1986, 1988a, b; Wollenweber et al. 1989). There are around 86 species growing in Chile (Marticorena and Quezada 1985; Ehrhart 2005) and only 15 % of them have been phytochemically characterized and less than 5 % will have done studies of biological activity.

Calceolaria integrifolia sensu lato complex comprises nine species: *C. andina, C. angustifolia, C. auriculata, C. georgiana, C. integrifolia s.str, C. rubiginosa, C. talcana, C. verbascifolia*, and *C. viscosissima.* These complex occur in Central-South Chile from V Region to VIII Region. Each one of these species has its own characteristic distribution pattern, which correlates with ecological and weather factors (Ehrhart 2005). Growing between the V to VIII region of Chile together with other species of *Calceolaria*. The most abundant are: *C. angustifolia, C. integrifolia, C. talcana* and *C. verbascifolia*, that are commonly known as "zapatito de doncella" or "capachito de hoja larga" these species are strong erect shrub, 150 cm tall or smaller with fragile ascending branches, internodes of 2–8 cm, and inflorescence and distal parts of stems glutinosus or velutines with erect hairs (Ehrhart 2005).

The taxonomy of *Calceolaria integrifolia s.l.* including many species classified in first time by Molau indicate:—Kingdom: Plantae—Phylum: Magnoliophyta—Class: Magnoliopsida—Order: Scrophulariales—Family: Calceolariaceae: Scrophulariaceae—Genus: *Calceolaria*—Species: *C.* spp. (for systematic see Molau 1988, 2003; Ehrhart 2000, 2005; Anderson 2006; Cosacov et al. 2009).

The phytochemical study of the extracts of *Calceolaria* species has led us to find a relationship between the extracts of *Penstemon gentianoides* and *P. campanulatus* (Plantaginaceae: Scrophulariaceae), both genus are formerly Scrophulariaceae and the phytochemicals found could be useful tools for chemotaxonomy studies on these species. *P. campanulatus* and *P. gentianoides* are evergreen shrubs that grows throughout high mountains from Guatemala, Mexico and Southern states of US. Its leaves and roots have been used therapeutically as anti-inflammatory and we have some reports about this activity (Domínguez et al. 2010) and antioxidant activity among others (Domínguez et al. 2005). Thus, in parallel manner similar phenylethanoids, iridoids and flavonoids have been identified in the aerial parts of *Calceolaria* (Cespedes et al. 2014; Domínguez et al. 2007; Muñoz et al. 2013a, b, c). Recently, we have reported an excellent review about the phytochemistry of *Calceolaria* species studied until now (Cespedes et al. 2013c) and additionally the absolute configuration of scopadulane diterpenoids in these species was reported (Muñoz et al. 2014).

In previous reports on the antifeedant, insect growth regulatory (igr) and insecticidal activities of *Calceolaria* species with a series of phenolic and terpene compounds, indicated that their maximum antifeedant and igr activity depends on the hydrophobic alkyl moieties from the hydrophilic hydroxyl groups (Cespedes et al. 2013c, 2014; Muñoz et al. 2013c). Additionally, their inhibitory effects on tyrosinase and acetylcholinesterase enzymes (Muñoz et al. 2013a, b, c; Cespedes et al. 2013a, b) and their function as proteinase inhibitors (Cespedes et al. 2014) was described.

Plant Defenses, constitutive or induced phytochemicals?

Plant–insect coevolution has been studied previously and several defense phytochemicals have been identified that plant species coevolved (Mithofer and Boland 2012; Ehrlich and Raven 1964; Berenbaum 1990). Thus some phytochemicals could be constitutive, or specifically induced after any pathogen attack (insect-herbivore or microorganism). In plants there are different ways of storing phytochemicals that are used for protection functions, such as vacuoles and trichomes. The glandular trichomes can exude toxic secretions such as phenolics or terpenoids (Bruckner et al. 2014; Colombo et al. 2014; Falcão et al. 2006; Kakuta et al. 1992; Kessler and Baldwin 2002; Mercado et al. 2010; Rhoades and Cates 1976; Woldemichael et al. 2003).

As a defense mechanism of *Calceolaria* complex against herbivore and phytopathogens attacks terpenes, phenolics and other compounds are accumulated along the aerial parts, mainly in flowers, leaves and trichomes, resulting in a unique biopesticides complex from these plants (Cespedes et al. 2013a, b, c, 2014; Muñoz et al. 2013c; Sachetti et al. 1999).

Until now pesticides of synthetic origin have been widely used, producing a strong impact on the environment with the development of strains resistant to this type of compound. Organic molecules of botanical origin may offer an environmentally safe source of compounds for pest management, since they are environmentally friendly and an efficient alternative to persistent organic pollutant-pesticides (Rhodes et al. 2013; Rosner and Markowitz 2013; Cespedes et al. 2014). The growing interest in the potential application of secondary metabolites in pest control leads to the search for new sources of biologically active natural products with low mammalian toxicity, low persistence in the environment, and biodegradability (Cao et al. 2013; González and Estevez-Braun 1998; Isman 2006). To date, global agricultural systems have consistently used pesticides of synthetic origin such as carbamates and organophosphorous (OP). These pesticides target acetylcholinesterase, which has resulted in the generation of new strains resistant to the original pesticides. Resistance development is related to the modification of receptors involved in the mechanisms and targets of action of certain molecules (Pang et al. 2012; Alout et al. 2012; Casida 2009; Casida and Durkin 2013). In response to the above-mentioned issue, the scientific community has synthesized a myriad of new synthetic organic molecules with this new target of action, which has resulted in negative health effects for other organisms

such as animals and humans. This pollution caused by synthetic agents in many countries on over the world is a serious problem that prompt our attention. Preventive measures in OP exposure are of great importance in human health in developing countries (Casida and Durkin 2013; Green et al. 2013; Fournier and Mutero 1994; Feyereisen 1995). Thus the search for new botanical pesticides (biopesticides) which have this target of action and remain harmless to animals and humans is relevant today. In the search of new weapons for the fight on the insect control and under a sustainably and friendly view on the environment it have centered the interest in the study of potential biocidal activities of herbs and shrubs belonging to the Calceolariaceae family, due to their strong resistance against pathogens attack observed in nature (Cespedes et al. 2013a, 2014).

Our initial attempt to clarify the mechanism of defense of this plant species against pathogen attack on a molecular level has been achieved slowly due to lack of availability of sufficient plant material of selected *Calceolaria* samples. Therefore, according to previous results obtained from the effects of extracts as insect growth inhibitors (Cespedes et al. 2013a, b, c; Muñoz et al. 2013a, b), is an important highlight and reinforce the characterization of new bioactive compounds from plant species belonging to *Calceolaria* samples from Americas reported until now.

These species are broadly used in Latin American Traditional Medicine for the treatment of antimicrobial, anti-inflammatory, digestive, diuretic and neurological diseases (Harborne and Baxter 2001; Mayhuasca et al. 2007; Muñoz et al. 2013a; Woldemichael et al. 2003). The Araucanian peoples used this plant for treating ''the nerves'' (an ethnomedical disease with symptoms of restlessness, insomnia, appetite loss, cardiac acceleration, and despair), for treating headache and inflammation (Montes and Wilkomirsky 1978; Muñoz et al. 2001) (the latter is intimately associated with AD), for dermatological uses in ethnomedicine, and as a tea for kidney health throughout South America (Montes 1987). Additionally, studies in *Calceolaria* species over the world show the presence of many substances with agrichemical applications and pharmacological potential (Falcão et al. 2006; Woldemichael et al. 2003; Muñoz et al. 2013c).

To date few studies have been performed investigating the phytochemical qualities and biological activities of *Calceolaria* species. Several studies have investigated the sites and mechanisms of action for fungicidal, insecticidal and/or insect growth regulatory activity shows that different secondary metabolites are enzymatic and metabolic inhibitors (Calderón et al. 2001; Cespedes et al. 2006; Feeny 1976) and have insecticidal, IGR and antifeedant effects on phytophagous insects (Rhoades and Cates 1976; Swain 1979; Simmonds et al. 1996; Xie et al. 1993; Ortego et al. 1995; Mullin et al. 1997; Muñoz et al. 2013c). We have previously demonstrated that diverse secondary metabolites have different sites of action and different molecular targets when they interact with enzymes and metamorphosis processes (Cespedes et al. 2006, 2013c, 2014). Because previously gathered information about this genus and our own field observations indicated that these plant species appear to possess a strong resistance to pathogen attack in the field, the subject of this review has been selected to be focused on the American members of *Calceolaria* species.

The general goal of this review is to update the information on the biological activities of *Calceolaria* species to establish the antimicrobial, antifeedant/insecticidal, anti-inflammatory, enzyme inhibitory and anticancer activity of some extracts and their isolates and also to measure the effect on metamorphosis of insect pest models. Several secondary metabolites from *Calceolaria* species have shown biocidal activities (Cespedes et al. 2013c; Falcão et al. 2007; Muñoz et al. 2013c; Woldemichael et al. 2003) and its occurrence in Calceolariaceae members has been reported previously in a detailed review by Cespedes group (Cespedes et al. 2013c). In continuation with our investigation this review about biological activities attempts to illuminate or shed light on the importance and relevance of studying plant species of the genus *Calceolaria*.

Biological activities

The bioactive isolates from a gradient between less polar (apolar) and higher polarity solvents extracts shows the presence of diterpenes, naphthoquinones, iridoids, phenylpropanoids and flavonoids, among others. Many of these samples has been assayed as antimicrobial (antibacterial and antifungal effects), insecticidal (against *D. melanogaster* (fruit fly) and *S.*

frugiperda (fall armyworm) mainly with aspects such as mortality, rate of development, time of pupation, adult emergence and deformities), and as anticancer activity (see Table 1).

Plants belonging to this family have been shown to possess interesting properties: aerial parts of these plants have medicinal activities that are used for digestive, diuretic and antimicrobial treatments (Harborne and Baxter 2001; Woldemichael et al. 2003). Several studies on *Calceolaria* species from around the world shows that this genus is characterized by the presence of substances with important agrochemical and pharmacological potential such as insecticide (Khambay et al. 1999) and antimicrobial properties (Falcão et al. 2006; Woldemichael et al. 2003).

The types of secondary metabolites that are mostly been reported in the literature correspond to diterpenes together with phenylpropanoids, flavonoids and phenolic compounds in general. Although endogenous functions of diterpenes have not yet been fully elucidated, these play important roles within the plant, especially in cell energy, and these types of substances have been shown to have important biological function in defensive activity.

They can to work both as constitutive and inducible defense mechanisms, also in dormancy and growth

Table 1 Reported biological activities of *Calceolaria* species

Species	Biological activity	Reference
C. thyrsiflora	Antitumoral: DU-145 (androgen-insensitive prostate cancer cells)	
	KB (oral squamous carcinoma cells)	Garbarino et al. (2007)
	Antibacterial	Bravo et al. (2005)
C. sessilis	Antitumoral: TA3 (mouse mammary adenocarcinoma cells)	
	TA3-MTX-R (methotrexate-multiresistant-subline)	Morello et al. (1995)
	Anti-Chagas: *Trypanosoma cruzi* (epimastigotes)	
C. andina	Insecticidal: inhibition of Complex III of the mitochondrial respiratory chain	Khambay et al. (1999), Khambay and Jewess (2000)
	Insecticidal: *Trialeurodes vaporariorum, Encarsia formosa*	Simmonds et al. (2002)
C. talcana	Insecticidal: *Spodoptera frugiperda, Drosophila melanogaster*	Cespedes et al. (2013a, b, c), Muñoz et al. (2013a, b)
	Cholinesterase inhibitor: Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE)	Cespedes et al. (2013a, b, c)
C. integrifolia	Insecticidal: *Spodoptera frugiperda, Drosophila melanogaster*	Cespedes et al. (2013a, b, c)
	Cholinesterase inhibitor: Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE)	
C. integrifolia s.l.	Antibacterial (*E. coli*, *E. agglomerans*, *B. subtilis*, *S. aureus*)	Cespedes et al. (2014)
	Antifungal (*R. solani*, *F. sporotrichum*, *F. moniliforme*, *A. niger*, *T. mentagrophytes*)	Cespedes et al. (2014)
C. tripartita	Dermatitis	Tene et al. (2007)
C. bicolor	Alleviate stomachaches	De la Cruz et al. (2007)
C. lobata	Alleviate stomachaches	De la Cruz et al. (2007)
C. engleriana	Fracture/sprains	Thomas et al. (2009)
C. parviflora	Fracture/sprains	Thomas et al. (2009)
C. buchtieniana	Cystitis, Kidney and vesicular ailments, Pains after childbirth, Prostate disorders	Macía et al. (2005)
C. incarum	Headache, migraña, Stomachache	de la Cruz et al. (2014)
C. linearis	Bath against cold	de la Cruz et al. (2014)
C. chelidonioides	Antibacterial, antioxidante	Falcão et al. (2006)
C. pinifolia	Antibacterial diterpenes	Woldemichael et al. (2003)
C. spp.	Trypanocidal	Leon et al. (2009)

inhibition of plants (Cespedes et al. 2006; Feeny 1976; Heath et al. 2014; Sachetti et al. 1999), in pest-resistance and antimicrobial activities, and as storage in trichomes (Kakuta et al. 1992; Mercado et al. 2010; Sachetti et al. 1999).

Against microorganisms

From the Argentinian *C. pinifolia* several interesting diterpenes with a promissory antibacterial activity were isolated. Samples of aerial parts were extracted with CH_2Cl_2/MeOH (1:1) mixture and further analyzed by HPLC in which 12 compounds including diterpenes of two new isopimaranes, 19-methylmalonyloxy-ent-isopimara-8(9),15-diene and 19-malonyloxy-entisopimar-a- 8(9),15-diene were isolated. Afterwards, using bioassay-guided fractionation of the CH_2Cl_2-MeOH (1:1) extract of the aerial parts of *C. pinifolia* eight other diterpenes and two triterpenes were isolated (Woldemichael et al. 2003). These compounds were assayed against *Staphylococcus aureus* (SA), methicillin resistant *S. aureus* (MRSA), *Bacillus subtilis* (BS), and *Escherichia coli* (EC). 4-Epi-dehydroabietinol and ent-isopimara-9(11),15-diene-19-ol were found to be active against MRSA with MIC values of 8 and 2 μg/ml, respectively. Mechanistic studies of ent-isopimara-9(11),15-diene-19-ol in BS suggested rapid and non-specific inhibition of uptake and incorporation of radiolabeled precursors into DNA, RNA, and protein consistent with membrane-damaging effects in bacteria, ent-isopimara-9(11),15-diene-19-ol did not afford protection against an acute infection with SA in mice (Woldemichael et al. 2003). On the other hand, among the diterpenes from *C pinnifolia*, 19-malonyloxydehydroabietinol and 19-methylmalonyloxy-ent-isopimara-8(9),15-diene was showed be active against *Mycobacterium tuberculosis* each with an MIC value of 4 μg/ml. MIC values for the triterpenes 3-epi-ursolic acid and 3-epi-oleanolic acid from *C. pinnifolia* were determined to be 8 and 16 μg/ml, respectively (Woldemichael et al. 2003).

From *C. thyrsiflora* Graham the antibacterial activity of DIBOA, HBOA, BOA, gallic acid and infusions of leaves and flowers were determined (Bravo et al. 2005). DIBOA show bacterial inhibitions reaching 100 % for *S. aureus*, 49.2 % for *E. coli* and 77 % for *S. mutans* at the highest concentration tested (1,000 μg/ml). At lower dose range than 250 μg/ml the inhibition decrease to 0 % for *S. aureus* and *E. coli* and 16 % for *S. mutans*. Only gallic acid showed a significant activity against *S. aureus* (67 % of inhibition) at lower dose. An opposite result was obtained when the antibacterial activity was assayed against *S. aureus* and *E. coli* by dispensing into petri dry surface of the agar disks (Feijo de Souza et al. 2004). Infusions of flowers display 100 % inhibition against *S. aureus* and *S. mutans* and the activity of infusions of leaves was lower in both bacteria. DIBOA, BOA and HBOA showed significant activity against *S. mutans*. The order of potencies DIBOA > BOA > HBOA was observed at the different concentrations. *S. mutans* is the most commonly cariogenic bacterium in humans. Therefore, these results represent a new interesting phytochemical subject of the medicinal properties of *C. thyrsiflora* Graham (Bravo et al. 2005).

Together with their antioxidant and antimicrobial activity, extracts from the aerial parts of *C. chelidonioides* were evaluated for their antibacterial (*Staphylococcus aureus* MRSA, *S. epidermidis* and *Staphylococcus aureus*) and antifungal (*Candida albicans, Cryptococcus neoformans, Fonsecaea pedrosoi* and *Trichophyton rubrum*) activities. EtOH extracts from stems, roots and hexane partition of flowers showed no activity at opposite microorganisms testing. All other extracts and partitions showed activity against the methicillin resistant strain of *S. aureus* (MRSA), the result of the extract flowers higher than the control and the leaves, similar done to control the concentration of vancomycin 1 mg/ml. This activity is of great importance due the high degree of pathogenicity and resistance of this strain. MRSA is commonly found in hospitals, and the principal in cases of hospital infection (Kantzanou et al. 1999). Although the ethanol extract of flowers have been presented the best results among the tested, this activity was reduced to be compared with your partitions, indicating that possibly the metabolites responsible for the activity are more polar and is retained in the partition butanolic not tested or due to synergism between the different substances present in the ethanol extract total (Cespedes et al. 2014; Woldemichael et al. 2003).

In a similar manner, different chemical structures of secondary metabolites have been isolated from *C. integrifolia* s.l. The chemical profile of selected members of the Chilean *C. integrifolia* s.l. complex represents a significant addition to previous studies.

Thus, bactericidal and fungicidal activities were determined. Dunnione mixed with gallic acid was the most active fungistatic and fungicidal combination encountered. Several compounds as isorhamnetin, combined with ferulic and gallic acid quickly reduced cell viability, but cell viability was recovered quickly and did not differ from that of the control. The effect of these mixtures on cultures of *Aspergillus niger*, *Fusarium moniliforme, Fusarium sporotrichum, Rhizoctonia solani*, and *Trichophyton mentagrophytes*, was sublethal. However, when fungistatic isorhamnetin and dunnione were combined with sublethal amounts of both ferulic and gallic acid, respectively, strong fungicidal activity against theses strains was observed. Thus, dunnione combined with gallic acid completely restricted the recovery of cell viability. This apparent synergistic effect was probably due to the blockade of the recovery process from induced-stress (Cespedes et al. 2014). The same series of phenolics (iridoids, flavonoids, naphthoquinones and phenylpropanoids) were also tested against the Gram-negative bacteria *Escherichia coli*, *Enterobacter agglomerans*, and *Salmonella typhi*, and against the Gram-positive bacteria *Bacillus subtilis, Sarcinia lutea*, and *Staphyllococcus aureus* and their effects compared with those that of kanamycin. Mixtures of isorhamnetin/dunnione/kaempferol/ferulic/gallic acid in various combinations were found to have the most potent bactericidal and fungicidal activity with MFC between 10 and 50 μg/ml. Quercetin was found to be the most potent fungistatic single compound with an MIC of 15 mg/ml. A time-kill curve study showed that quercetin was fungicidal against fungi assayed at any growth stage. This antifungal activity was slightly enhanced by combination with gallic acid. The primary antifungal action of the mixtures assayed likely comes from their ability to act as nonionic surfactants that disrupt the function of native membrane-associated proteins. Hence, the antifungal activity of isorhamnetin and other *O*-methyl flavonols appears to be mediated by biophysical processes. Maximum activity is obtained when the balance between hydrophilic and hydrophobic portions of the molecules of the mixtures becomes the most appropriate. Diterpenes, flavonoids, phenylpropanoids, iridoids and phenolic acids were identified by chromatographic procedures (HPLC–DAD), ESI–MS, and NMR hyphenated techniques (Cespedes et al. 2014).

Against insects: plant–insect herbivore interaction

Several studies have investigated the sites and mechanisms of action of allelochemicals, and insecticidal or insect growth inhibitory (IGR) activity shows that different diterpenes are enzymatic and metabolic inhibitors (Calderón et al. 2001; Cespedes et al. 2006; Feeny 1976) and have insecticidal, IGR and antifeedant effects on phytophagous insects (Rhoades and Cates 1976; Simmonds et al. 1996; Xie et al. 1993; Ortego et al. 1995; Mullin et al. 1997). It have previously demonstrated that diverse secondary metabolites have different sites of action and different molecular targets when they interact with enzymes and metamorphosis processes (Cespedes et al. 2006).

To date, few studies have been performed investigating the phytochemical qualities or biological activities of many *Calceolaria* sp.pl. Because previously gathered information about this genus and our own field observations indicated that these plant species appear to possess a strong resistance to pathogen attack. Cespedes' group undertook examination of Chilean members of this genus specifically the *C. integrifolia* s. l. complex (Cespedes et al. 2013b, c, 2014; Muñoz et al. 2013a, b, c) whose long-term goal is to learn about the role of the phytochemical composition with the inhibitory behavior on growth and development of a model system of pest insect such as *Drosophila melanogaster* (Diptera: Drosophilidae) and *Spodoptera frugiperda* (J.E. Smith, Lepidoptera: Noctuidae). In short, they are in search of botanicals for potential use as biopesticides. All these data is important for the studies on insect control (Singh-Ratan 2010).

Despite the few studies of biological activities of the secondary metabolites isolated from *Calceolaria* species, these have been shown to be antifeedant, tyrosinase inhibition, antibacterial, anticancer, antioxidant, and trypanocidal (Bravo et al. 2005; Cespedes et al. 2013a, b, c; Falcão et al. 2006; Khambay et al. 1999; Morello et al. 1995; Muñoz et al. 2013a, b, c; Woldemichael et al. 2003)

Many interactions are occurring at the environment where are associated plant and insects. The plant–insect interactions can reveal evolved plant defenses and insect counter adaptations that often are missing by prolonged periods of coevolution. In this review we do an approximation to understand and to utilize this

interaction to explore *Calceolaria* defenses and pathogen adaptations in a new light.

Exudates collected from three species (*C. andina, C. integrifolia* and *C. talcana*) was extremely toxic to fungi, bacteria and generalists *S. frugiperda* and *D. melanogaster*, thus providing *Calceolaria* with a potent defense that prevents phytopathogens attack (Cespedes et al. 2014). Glandular trichomes were specifically extracted, concentrated and assayed for toxic effects showing high mortality, fungicidal and antibacterial activity (Cespedes et al. 2013c, 2014; Muñoz et al. 2013c).

Final instar fall army worm (*S. frugiperda*), and fruit fly (*D. melanogaster*) were tested in laboratory assays to determine if a particular *Calceolaria* chemical induces insect growth regulatory behavior (Cespedes et al. 2013c, 2014; Muñoz et al. 2013c).

In order to determine a possible correlation between insect growths regulatory (IGR), acute toxicity, and ecdysis caused by these isolates, oral injection of all samples at different concentrations into ten larvae of 21 days of *S. frugiperda* was carried out. Concentrations promoted apolysis to the fifth instar, but inhibited ecdysis, whereas oral injection of *n*-hexane extract resulted only in a delay of the normal molt to the fifth instar. At higher concentrations, after 48 and 72 h prothetely the appearance of precocious pupal structures in the larvae was induced, in some (30 %) of the treated fourth instar larvae, these larvae molted directly to pupae (Cespedes et al. 2013a, b, c; Muñoz et al. 2013a, b, c). Sample-induced prothetely resulted in precociousness and browning of pupae in roughly half of the controls, these effects could be observed with phytoecdysteroids analogues from other plants (Cespedes et al. 2006). Additionally, prothetely can be elicited by application of juvenile hormone or juvenile hormone mimics (Truman and Riddiford 2002).

Antioxidant, tyrosinase, acetylcholinesterase and Protease Inhibitory Activity

Several compounds and mixtures assayed showed strong acute toxicity with a potent acute toxicity of survival on larvae of last stage of *S. frugiperda*. Preceding experimental observations suggest that acute toxicity and growth inhibition of our samples may be due to inhibition of a proteinase, ETH and other polyphenol oxidases (PPO) that could bind to these compounds. These targets have been demonstrated for other compounds of natural origin (Kessler and Baldwin 2002).

The insect growth inhibitory activity showed by the extracts and compounds from *C. integrifolia* s.l. (Cespedes et al. 2013a, b, c; Muñoz et al. 2013a, b, c) seems to correlate with the tyrosinase inhibitory activity, but it should be borne in mind that plant secondary metabolites can act by a variety of different mechanisms in insects such as proteinase inhibitors (Jongsma and Bolter 1997; Farmer and Ryan 1990). The sensitivity of insect pest models to ingestion of phenylpropanoids (verbascoside and analogues) and naphthoquinones (dunnione and analogues) may be a consequence of the extensive chemical modification in the midgut by oxidation. If so, naphthoquinones should be the first oxidized structure. Quinones are usually toxic to insects as well as to many other organisms (Khambay et al. 1999, 2003; Akhtar et al. 2012).

However, it should be noted that benzoic acid in *Calceolaria* species inhibited tyrosinase, but did not inhibit insect growth. This result may support that the oxidized products are responsible for the activity. Radical scavenging activity, which can be measured as decolorizing activity following trapping of the unpaired electron of DPPH, was examined. The possibility that their adverse effects are a consequence of their potential to act as a prooxidant should also be considered. In fact, gallic acid, under certain conditions, can produce superoxide anion (Serrano et al. 1998).

Furthermore, hydroxynaphthoquinone may irreversibly inactivate enzymes (proteins) in the midgut, a process known as tanning, prior to being oxidized. This process also needs to be taken into consideration, as hydroxynaphtoquinone may bind with proteins in the gut and, as a result, inhibit digestive enzymes as well as protein digestion (Ryan 1979). This can be supported by the observation that hydroxynaphthoquinones showed significant inhibitory activity against fungal protease. At a concentration of 10 μg/ml, naphthoquinone inhibited enzymatic activity 68 %, while dunnione did not exhibit any inhibitory activity up to 75 μg/ml. In addition, naphthoquinone inhibited the assayed digestive enzymes (Type II fungal protease of *Aspergillus oryzae* and casein powder). Dunnione also inhibited these enzymes but much more weakly compared to hydroxynaphthoquinone. This

fact shows that the addition of a hydroxyl group resulted in a significant increase in the toxicity (Akhtar el al. 2012). On the other hand, dunnione did not show insecticidal activity, although it has an unusually high activity on fungal proteinase at concentrations higher than 75 μg/ml (>90 % at 250 μg/ml) (Muñoz et al. 2013c; Cespedes et al. 2014), similar to findings of Khambay et al. (2003), and Li et al. (2014).

Digestive proteases catalyze the release of peptides and amino acids from dietary protein, and they are found most abundantly in the midgut region of the insect digestive tract (Passi and Nazzaro-Porro 1981). Different proteases can be inhibited by many secondary metabolites from the plants; this process can be termed proteinase inhibitor inducing factor (PIIF) (Ryan 1979; Guerrero and Rossel 2005). The more polar fractions of the aerial parts (dried stems and leaves) of the plant species *C. integrifolia* s.l. yielded the most active compounds (verbascoside and 2-hydroxy-3-(1,1-dimethylallyl)-1,4-naphthoquinone), which inhibited tyrosinase as well as two digestive proteinases tested that were used as a protease enzyme model, than those of medium or low polarity.

Then, it may be logical to assume that phenolics such as verbascoside and the hydroxynaphthoquinone for example are synthesized during the preliminary attacks on *C. integrifolia* s.l. by an unidentified coleopteran insect observed on this plant, which after several hours searches for another plant (Muñoz et al. 2013c). It appears therefore that the releasing mechanism of phenolics may be one of the key processes to understanding plant defense (Hutcheson 1998; Singh et al. 2001; Yamane et al. 2010).

Anticancer activity

Demalonyl thyrsiflorin A, a semisynthetic labdane-derived diterpenoid, induces apoptosis and necrosis in human epithelial cancer cells. Thyrsiflorin A, a diterpene with the scopadulane skeleton was isolated from *C. thyrsiflora*. Experimental evidences on the semisynthetic analogues of scopadulane diterpenes have permitted to hypothesize that a polar substituent is important for the antitumor activity of this class of compounds. Therefore, the effect of the semisynthetic compound, demalonyl thyrsiflorin A, on cell growth and death in two human epithelial cell lines, DU-145 cells (androgen-insensitive prostate cancer cells) and KB cells (oral squamous carcinoma cells) was investigated. The results indicated that this compound, exhibited comparable degrees of antigrowth effect on cancer cells examined as judged by IC_{50} values, 9.77 μM (2.73 μg/ml) and 10.86 μM (3.04 μg/ml) in DU-145 and KB cells, respectively, and support the hypothesis that also for diterpenoid compounds an available hydroxyl group is important for decreased cancer cell viability. In addition, it was demonstrated an apoptotic response after treatment of DU-145 and KB cells with this semisynthetic compound at 6-12 μM concentrations, together with a necrosis process at higher doses (25-50 μM). Both apoptotic and necrotic pathway implicated in demalonyl thyrsiflorin A-treated cells are correlated with the elevation of ROS (reactive oxygen species) generation (Garbarino et al. 2007).

The naphthoquinones 2-hydroxy-3-(l,l-dimethylallyl)-l,4-naphthoquinone (A) (-)-2,3,3-trimethyl-2-3-dihydronaphtho[2,3-b]furan-4,9-quinone (B), and 2-acetoxy-3-(l,l-dimethylallyl)-1,4-naphthoquinone (C) isolated from *Calceolaria sessilis* were tested against *Trypanosoma cruzi* epimastigotes, the TA3 tumor cell line and the methotrexate-resistant subline TA3-MTX-R. Naphthoquinone C was the most active; the 50 % culture growth inhibition (I_{50}) on *T. cruzi* (Tulahuen and LQ strain and DM28c clone) was at concentrations ranging from 2.1 to 5.2 μmolar. Also compound C inhibited TA3 and TA3-MTX-R culture growth with an I_{50} of 2.1 and 3.8 μmolar, respectively. Also naphthoquinone C inhibited the respiration of the tumor cells by interfering with the electron transport at some point between NADH and ubiquinone. The respiration of *T. cruzi* was not inhibited by naphthoquinone C. Naphthoquinone C produced a temporary increase of oxygen consumption in *T. cruzi* and tumor cells, suggesting the generation and participation of free radicals (Morello et al. 1995). These effects can be correlated with new findings on naphthoquinones with anticancer activity (Takemoto et al. 2014) that provide new insights about its site and mode of action.

C. chelidonioides is an original Brazilian plant belonging to Calceolariaceae, which is used in the Brazilian folk medicine for the treatment of several kinds of cancer (Falcão et al. 2007). Its cytotoxicity, phototoxicity and genotoxicity potential were evaluated in different methodologies in vitro using the flowers ethanol extract. The cytotoxicity and phototoxicity were evaluated by the neutral red dye assay

using keratinocyte human cells (NCTC 2544). For the phototoxicity evaluation the cell culture containing the compounding test was submitted to UVA radiation (345 nm) during 15 min. The assays showed the cell viability after the treatment with the extract and its metabolites formed by the UV radiation. The genotoxicity potential was evaluated by the Comet assay using keratinocyte human cells incubated for 1 h with the extract, with and without metabolic activation using the S9 mix. The Comet assay is able to detect different kinds of DNA fragmentations caused by the genotoxic agents. The cells which DNA were damaged show an image comet like with a "head" and a "tail" that elongates proportionally to the DNA damages. A second method used was the Ames' test which is capable to detect compounds with carcinogenic and mutagenic properties using mutants *Salmonella typhimurium* strains to detect base substitution and frame shift point mutations. These tests were also evaluated with and without metabolic activation. The *Calceolaria* flowers ethanol extract or even its metabolites didn't show any kind of toxicity in all tested models (Falcão et al. 2007).

Concluding remarks

The sites, mechanisms and mode of action of these extracts, mixtures and compounds are continuing to be investigated and probably correspond to a combination of different antifeedant actions, which, when considered at the molecular level, could have the following targets: midgut phenol oxidase, proteinase, ETH, tyrosinase or other PPOs and cuticle synthesis inhibition, as well as molting sclerotization toxicity, as has been found for other natural compounds (Akhtar and Isman 2013; Kubo et al. 2003a, b; Kubo 1997; Cespedes et al. 2000, 2006; Torres et al. 2003) and extracts (Feng et al. 1995).

The results reported until now demonstrate that *Calceolaria* species herbs and shrubs that growth on natural, perturbed and semi-perturbed lands after are harvested native and/or pine forests or together with pine trees, irrespective of whether they have co-evolved or not with particular insect herbivores (Moreira et al. 2013). The biotic stimuli needed to elicit specific induced responses may include recognition of the enemies—herbivore-associated (Mithofer and Boland 2012). Moreover, the biotic stimuli could (may) influence-induce specific combination of volatile compounds together with interactive effects triggering biosynthesis of chemical defenses (Marin and Cespedes 2007). This scope has mainly been investigated in pathogen-insect vector-plant interactions (Zhu et al. 2014) and could be usefulness in food industry (Capanoglu 2010).

In summary, the insecticidal activity of extracts from aerial parts from *Calceolaria* species may be due to a synergistic effect shown by the ecdysone-like activity of the extracts in the test system used in this investigation.

The results suggest that insect growth inhibitory activity of mixtures and compounds could be caused not only by a strong inhibitor suggesting a synergistic effect of the composition of the mixture. In addition to antimicrobial agents and enzyme inhibitors, the significant inhibition of insect growth by these compounds shows that these samples may be considered as efficient IGR.

Finding alternative insect control agents by searching for plant compounds that inhibit tyrosinase is one of the goals of our continuing research. However, in vitro results using fungal tyrosinase as described are still far from our goal. Mushroom tyrosinase used for the initial screening differs somewhat from that of insects. More importantly, it has not been intentionally overlooked, but, as a result, the dynamic function of tyrosinase in insect cuticle formation has not been thoughtfully taken into account. Thus, tyrosinase does not always exist as the active form in insects, and tyrosinase inhibitors cannot always reach the cuticle in sufficient concentrations to be effective. In addition, the reaction time and amount of available oxygen need to be considered from a practical point of view, as insect tyrosinase is an aerobic oxidase. As far as the artificial diet feeding assay against *S. frugiperda* and *D. melanogaster* larvae is concerned, some tyrosinase inhibitors characterized inhibit insect growth but some do not. The fact that plant secondary metabolites function by a variety of different mechanisms in insects needs to be kept in mind.

The relevance of the results of in vitro experiments in simplified systems to the in vivo situation should be carefully considered. The naphthoquinones remains as potential antimicrobial (Li et al. 2014) and anticancer substances from this plant species. We are doing further studies using more appropriate bioassay methods.

Acknowledgments The authors are indebted to M.Sc. Evelyn Muñoz (Phytochemical Ecology Lab, Basic Sciences Dept., Faculty of Sciences, University of Bio Bio, Chillan, Chile) for technical assistance with the antioxidant assay and for the help in performing many insect feeding assays. To Prof. Ana Ma. Garcıa-Bores (Laboratorio de Fitoquimica, Unidad UBIPRO, FES-Iztacala, UNAM, Mexico DF, Mexico) for proteinase assays and performing antimicrobial assay in part. To Prof. David S. Seigler, Ph.D. (Emeritus Professor, Department of Plant Biology, and Curator, Herbarium of University of Illinois at Urbana-Champaign) for identification of the plant samples. CLC is grateful to CONICYT Chile through FONDECYT Program, grants # 1101003 and # 1130242 for financial support in part. IK and CLC are grateful to UC Berkeley-Chile Seed Funds for grant. This paper is based on work supported by grants from the Comision Nacional de Investigacion Cientifica y Tecnologica de Chile (CONICYT), through FONDECYT Program grants 1101003 and 1130242.

References

Akhtar Y, Isman MB (2013) Plant natural products for pest management: the magic of mixtures. In: Ishaaya I, Reddy Palli S, Rami Horowitz A (eds) Advanced technologies for managing insect pests. Springer, Netherland, pp 231–247

Akhtar Y, Yeoung YR, Isman MB (2008) Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. Phytochem Rev 7:77–88

Akhtar Y, Isman MB, Lee Ch-H, Lee S-G, Lee H-S (2012) Toxicity of quinones against two-spotted spider mite and three species of aphids in laboratory and green house conditions. Ind Crops Prod 37(1):536–541

Alout H, Labbe P, Berthomieu A, Djogbenou L, Leonetti J-P, Fort Ph, Weill M (2012) Novel AChE inhibitors for sustainable insecticide resistance management. PLoS-ONE 7(10): e47125,1–8. doi:10.1371/journal.pone.0047125

Anderson S (2006) On the phylogeny of the genus *Calceolaria* (Calceolariaceae) as inferred from ITS and plastid *matK* sequences. Taxon 55(1):125–137

Berenbaum MR (1989) North American ethnobotanicals as sources of novel-plant based insecticides. In: Arnason JT, Philogene BJR, Morand P (eds) Insecticides of plant origin. ACS symposium series. American Chemical Society, Washington, vol 387, pp 11–24)

Berenbaum MR (1990) Evolution of specialization. In insect-umbellifer associations. Annu Rev Entomol 35:319–343

Berenbaum MR (2002) Postgenomic chemical ecology: from genetic code to ecological interactions. J Chem Ecol 28(5):873–896

Bowers MD (1981) Unpalatability as a defense strategy of western checkerspot butterflies (Euphydrias Scudder, Nymphalidae). Evolution 35(2):367–375

Bravo HR, Copaja SV, Figueroa-Duarte S, Lamborot M, San Martin J (2005) 1,4-Benzoxazin-3-one, 2-benzoxazolinone and Gallic acid from *Calceolaria thyrsiflora* Graham and their antibacterial activity. Z Naturforsch C 60(5/6):389–393

Bruckner K, Bozic D, Manzano D, Papaefthimiou D, Pateraki I, Scheler U, Ferrer A, de Vos RCH, Kanellis AK, Tissier A (2014) Characterization of two genes for the biosynthesis of abietane-type diterpenes in rosemary (*Rosmarinus officinalis*) glandular trichomes. Phytochemistry 101:52–64

Calderón JS, Cespedes CL, Rosas R, Gomez-Garibay F, Salazar JR, Lina L, Aranda E, Kubo I (2001) Acetylcholinesterase and insect growth inhibitory activities of *Gutierrezia microcephala* on fall armyworm *Spodoptera frugiperda* J. E. Smith. Z. Naturforschung 56c: 382–394

Cao J, Xia X, Dai X, Xiao J, Wang Q, Andrae-Marobela K, Okatch H (2013) Flavonoids profiles, antioxidant, acetylcholinesterase inhibition activities of extract from Dryoathyrium boryanum (Willd.) Ching. Food Chem Toxicol 55:121–128

Capanoglu E (2010) The potential of priming in food production. Trends Food Sci Technol 21:399–407

Casida JE (2009) Pest toxicology: the primary mechanisms of pesticide action. Chem Res Toxicol 22:609–619

Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Ann Rev Entomol 58:99–117

Cespedes CL, Calderón J, Lina L, Aranda E (2000) Growth inhibitory effects on fall armyworm Spodoptera frugiperda of some limonoids isolated from Cedrela spp. (Meliaceae). J Agric Food Chem 48:1903–1908

Céspedes CL, Alarcón J, Aranda E, Becerra J, Silva M (2001a) Insect growth regulatory and insecticidal activity of b-dihydroagarofurans from Maytenus spp. (Celastraceae). Z Naturforsch C 56c:603–613

Céspedes CL, Martínez-Vázquez M, Calderón JS, Salazar JR, Aranda E (2001b) Insect growth regulatory activity of some extracts and compounds from Parthenium argentatum on fall armyworm Spodoptera frugiperda. Z Naturforsch C 56c:95–105

Cespedes CL, Avila JG, Marin JC, Domínguez M, Torres P, Aranda E (2006) Natural compounds as antioxidant and molting inhibitors can play a role as a model for search of new botanical pesticides. In: Rai M, Carpinella MC (eds) Naturally occurring bioactive compounds. Advances in phytomedicine series, vol 3. Elsevier, The Netherlands, pp 1–27

Cespedes CL, Muñoz E, Lamilla L, Molina SF, Alarcon J (2013a) Insect growth regulatory, molting disruption and insecticidal activity of *Calceolaria talcana* (Calceolariaceae: Scrophulariaceae) and *Condalia microphylla* Cav@ (Rhamnaceae), Chapter 15. In: Cespedes CL, Sampietro DA, Seigler DS, Rai MK (eds) Natural antioxidants and biocides from wild medicinal plants. Cabi Publishing, Wallingford

Cespedes CL, Muñoz E, Salazar JR, Yamaguchi L, Werner E, Alarcón J, Kubo I (2013b) Inhibition of cholinesterase activity by extracts, fractions and compounds from *Calceolaria talcana* and *C. integrifolia* (Calceolariaceae: Scrophulariaceae). Food Chem Toxicol 62:919–926

Cespedes CL, Salazar JR, Alarcón J (2013c) Chemistry and biological activities of *Calceolaria* spp. (Calceolariaceae: Scrophulariaceae). Phytochem Rev 12:733–749

Cespedes CL, Salazar JR, Ariza-Castolo A, Yamaguchi L, Avila JG, Aqueveque P, Kubo I, Alarcon J (2014) Biopesticides from plants: calceolaria integrifolia s.l. Environ Res 132:391–406

Colombo PS, Flamini G, Christodoulou MS, Rodondi G, Vitalini S, Passarella D, Fico G (2014) Farinose alpine primula

species: phytochemical and morphological investigations. Phytochemistry 98:151–159
Conner WE, Boada R, Schroeder, FC, Gonzalez A, Meinwald J, Eisner T (2000) Chemical defense: bestowal of a nuptial alkaloidal garment by a male moth on its mate. Proc Nat Acad Sci 97(26):14406–14411
Cosacov A, Sérsic AN, Sosa V, De-Nova JA, Nylinder S, Cocucci AA (2009) New insights into the phylogenetic relationships, character evolution, and phytogeographic patterns of *Calceolaria* (Calceolariaceae). Am J Bot 96(12):2240–2255
Crombie L (1999) Natural product chemistry and its part in the defense against insects and fungi in agriculture. Pestic Sci 55:761–774
De la Cruz H, Vilcapoma G, Zevallos PA (2007) Ethnobotanical study of medicinal plants used by the Andean people of Canta, Lima. Peru J Ethnopharmacol 111(2):284–294
De la Cruz MG, Malpartida SB, Santiago HB, Jullian V, Bourdy G (2014) Hot and cold: Medicinal plant uses in Quechua speaking communities in the high Andes (Callejón de Huaylas, Ancash, Perú). J Ethnopharmacol 155(2):1093–1117
Di Fabio A, Bruni A, Poli F, Garbarino JA, Chamy MC, Piovano M, Nicoletti M (1995) The distribution of phenylpropanoid glycosides in Chilean *Calceolaria* spp. Biochem Syst Ecol 23(2):179–182
Domínguez M, Nieto A, Marín JC, Keck A-S, Jeffery E, Cespedes CL (2005) Antioxidant activities of extracts from Barkleyanthus salicifolius (Asteraceae) and Penstemon gentianoides (Scrophulariaceae). J Agric Food Chem 53:5889–5895
Domínguez M, Marín JC, Esquivel B, Cespedes CL (2007) Pensteminoside, an unusual catalpol-type iridoid from Penstemon gentianoides HBK (Plantaginaceae). Phytochemistry 68:1762–1766
Domínguez M, Keck AS, Jeffery EH, Cespedes CL (2010) Effects of extracts, flavonoids and iridoids from *Penstemon gentianoides* (Plantaginaceae) on inhibition of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) in LPS-Activated RAW 264.7 macrophage cells and their antioxidant activity. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 9(5):397–413
Ehrhart Ch (2000) Die gattung *Calceolaria* (Scrophulariaceae) in Chile. Biblioth Bot H 153:1–283
Ehrhart Ch (2005) The Chilean *Calceolaria integrifolia* s.l. species complex (Scrophulariaceae). Syst Bot 30:383–411
Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608
Eisner T, Eisner M, Aneshansley DJ, Wu CL, Meinwald J (2000) Chemical defense of the mint plant, Teucrium marum (Labiatae). Chemoecology 10(4):211–216
Engler A (1964) Sillabus der Pflanzenfamilien. Melchio H (ed) Berlin, Borntrager, vol II, p 451
Falcão DQ, Costa ER, Alviano DS, Alviano CS, Kuster RM, Menezes FS (2006) Atividade antioxidante e antimicrobiana de *Calceolaria chelidonioides* Humb. Bonpl Kunth Braz J Pharmacog 16(1):73–76
Falcão DQ, Costa ER, Kuster RM, Nielloud F, Vian L, Menezes FS (2007) Evaluation of cytotoxicity, phototoxicity and genotoxicity from *Calceolaria chelidonioides* (Scrophulariaceae) flowers ethanol extract. Plant Med pp 73–77
Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci 87:7713–7716
Feeny PP (1976) Plant apparency and chemical defense. In: Wallace JW, Mansell RL (eds) Biochemical Interactions between Plants and Insects. Plenum Press, New York, pp 1–40
Feijo de Souza J, Carvalho M, Trevisan D, Schmitz W, Saridakis H (2004) Phenolic compounds and hydroxymethylfurfural from the flowers of Caesalpinia peltophoroides and their antibacterial activity. Rev Latinoamer Quim 32:25–29
Feng RY, Chen WK, Isman MB (1995) Synergism of malathion and inhibition of midgut esterase activities by an extract from Melia toosendan (Meliaceae). Pestic Biochem Physiol 53:34–41
Feyereisen R (1995) Molecular biology of insecticide resistance. Toxicol Lett 82(83):83–90
Fournier D, Mutero A (1994) Modification of acetylcholinesterase as a mechanism of resistance to insecticides. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 108(1):19–31
Garbarino JA, Chamy MC, Piovano M (2000) Chemistry of the *Calceolaria* genus. Structural and biological aspects. Molecules 5(3):302–303
Garbarino JA, Chamy MC, Piovano M, Espinoza L, Belmonte E (2004) Diterpenoids from *Calceolaria inamoena*. Phytochemistry 65(7):903–908
Garbarino JA, Cardile V, Lombardo L, Chamy MC, Piovano M, Russo A (2007) Demalonyl thyrsiflorin A, a semisynthetic labdane-derived diterpenoid, induces apoptosis and necrosis in human epithelial cancer cells. Chem Biol Interact 169(3):198–206
González JA, Estevez-Braun A (1998) Effect of *E*-chalcone on potato-cyst nematodes (*Globodera pallida* and *G. rostochiensis*). J Agric Food Chem 46:1163–1165
Gonzalez-Coloma A, Gutierrez C, Cabrera R, Reina M (1997) Silphinene derivatives: their effects and modes of action on Colorado potato Beetle. J Agric Food Chem 45:946–950
Green BT, Welch KD, Panter KE, Lee ST (2013) Plant toxins that affect nicotinic acetylcholine receptors: a review. Chem Res Toxicol 26(8):1129–1138
Guerrero A, Rosell G (2005) Biorational approaches for insect control by enzymatic inhibition. Curr Med Chem 12:461–469
Harborne J, Baxter H (2001) Chemical dictionary of economic plants. Wiley, Chichester
Heath JJ, Kessler A, Woebbe E, Cipollini D, Stireman JO (2014) Exploring plant defense theory in tall goldenrod, *Solidago altissima*. New Phytol 202:1357–1370
Hutcheson SW (1998) Current concepts of active defense in plants. Annu Rev Phytopathol 36:59–80
Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66
Isman MB, Akthar Y (2007) Plant natural products as a source for developing environmentally acceptable insecticides. In: Ishaaya I, Nauen R, Horowitz AR (eds) Insecticides design using advanced technologies. Springer, Berlin, pp 235–248
Isman MB, Grieneisen ML (2014) Botanical insecticide research: many publications, limited useful data. Trends Plant Sci 19(3):140–145
Jongsma MA, Bolter C (1997) The adaptation of insects to plant protease inhibitors. J Insect Physiol 43(10):885–895
Kakuta H, Seki T, Hashidoko Y, Mizutani J (1992) Ent-kaurenic acid and its related compounds from glandular trichome

exudate and leaf extracts of *Polymnia sonchifolia*. Bioscience Biotechnol Biochem 56:1562–1564

Kantzanou M, Tassios PT, Tseleni-Kotsovili A, Legakis LJ, Vatopoulos AC (1999) Reduced susceptibility to vancomycin of nosocomial isolates of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 43(5):729–731

Keane S, Ryan MF (1999) Purification, characterization, and inhibition by monoterpenes of acetylcholinesterase from the waxmoth, Galleria mellonella (L.). Insect Biochem Mol Biol 29:1097–1104

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Ann Rev Plant Biol 53:299–328

Khambay BPS, Jewess PJ (2000) The potential of natural naphthoquinones as the basis for a new class of pest control agents: an overview of research at IACR-Rothamsted. Crop Prot. 19(8/10):597–601

Khambay BPS, Batty D, Cahill M, Denholm I, Mead-Briggs M, Vinall S, Niemeyer HM, Simmonds MSJ (1999) Isolation, characterization, and biological activity of naphthoquinones from *Calceolaria andina* L. J Agric Food Chem 47(2):770–775

Khambay BPS, Batty D, Jewess PJ, Bateman GL, Holloman DW (2003) Mode of action and pesticidal activity of the natural product dunnione and of some analogues. Pest Manag Sci 59(2):174–182

Kubo I (1997) Tyrosinase inhibitors from plants. In: Hedin P, Hollingworth R, Masler E, Miyamoto J, Thompson D (eds) Phytochemicals for pest control. ACS symposium series 658, American Chemical Society, Washington, pp 310–326

Kubo I, Klocke A, Asano S (1981) Insect ecdysis inhibitors from the east African medicinal plant Ajuga remota (Labiatae). Agric Biol Chem 45:1925–1927

Kubo I, Muroi H, Himejima M (1993) Combination effects of antifungal nagilactones against Candida albicans and two other fungi with phenylpropanoids. J Nat Prod 56:220–226

Kubo I, Kinst-Hori I, Chauduri SK, Kubo Y, Sánchez Y, Ogura T (2000) Flavonols from Heterotheca inuloides: tyrosinase inhibitory activity and structural criteria. Bioorg Med Chem 8:1749–1755

Kubo I, Chen Q-X, Nihei KI, Calderón JS, Cespedes CL (2003a) Tyrosinase inhibition kinetics of anisic acid. Z Naturforsch C 58c:713–718

Kubo I, Kinst-Hori I, Nihei KI, Soria F, Takasaki M, Calderón JS, Cespedes CL (2003b) Tyrosinase inhibitors from galls of Rhus javanica leaves and their effects on insects. Z Naturforsch C 58c:719–725

Leon E, Guillen Z, Felix LM, Chavez J, Martinez R (2009) Parasitemy of *Trypanosoma cruzi* in *Mus musculus* and preliminary in vitro study of the trypanocidal action of two species of *Calceolaria* in Peru. Revista de Investigacion de la Universidad Norbert Wiener, year 2009:81–88

Li Sh, Shao M-W, Lu Y-H, Kong L-Ch, Jiang D-H, Zhang Y-L (2014) Phytotoxic and antibacterial metabolites from *Fusarium proliferatum* ZS07 isolated from the gut of long-horned grasshoppers. J Agric Food Chem 62:8997–9001

Macía MJ, García E, Vidaurre PJ (2005) An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol 97(2):337–350

Marin JC, Cespedes CL (2007) Compuestos volatiles de plantas. Origen, emisión, efectos, análisis y aplicaciones al agro [Volatile compounds from plant. Origin, emissions, effects, analysis and agro applications]. Rev Fitotec Mex 30(4):327–351

Mayhuasca O, Bonilla P, Ballon M, Ferreira M, Carrasco E (2007) Efecto antiinflamatorio en ratones de extractos de *Calceolaria tripartita* R&P, comparación fitoquimica con el extracto hidroalcoholico de *Calceolaria melissifolia* Bentham [Antiinflammatory effect in mice of extracts of *Calceolaria tripartita* R&P, compared phytochemical with the hydroalcoholic extract of *Calceolaria melissifolia* Bentham. Ciencia e Investigacion [Facultad de Farmacia y Bioquimica, UNMSM] 10(2): 71–80

Meinwald J (2001) Sex, violence and drugs in the world of insects: a chemist's view. Science 5:80–92

Marticorena C, Quezada M (1985) Catalogo de la flora vascular de Chile. Gayana Bot 42:1–157

Mercado MI, Coll MV, Grau A, Catalan CAN (2010) New acyclic diterpenic acids from Yacon (Smallanthus sonchifolius) leaves. Nat Prod Commun 5:1721–1726

Miresmailli S, Isman MB (2014) Botanical insecticides inspired by plant-herbivore chemical interactions. Trends Plant Sci 19(1):29–35

Mithofer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450

Molau U (1988) Scrophulariaceae: part I. Calceolarieae. Flora Neotropica 47:1–326

Molau U (2003) Two new species of Calceolaria (Scrophulariaceae) from the tropical Andes. Novon 13:101–103

Montes M (1987) Aspectos de la medicación popular en la región del Bío Bío. Acta Bonaerense 6:115–124

Montes M, Wilkomirsky T (1978) Plantas chilenas en medicina popular: Ciencia y Folklore. Universidad de Concepción, Concepción, Chile, Escuela de Química y Farmacia y Bioquímica

Moreira X, Lundborg L, Zas R, Carrillo-Gavilan A, Borg-Karlson AK, Sampedro L (2013) Inducibility of chemical defenses by two chewing insect herbivores in pine trees is specific to targeted plant tissue, particular herbivore and defensive trait. Phytochemistry 94:113–122

Morello A, Pavani M, Garbarino JA, Chamy MC, Frey C, Mancilla J, Guerrero A, Repetto Y, Ferreira J (1995) Effects and mode of action of 1,4-naphthoquinones isolated from *Calceolaria sessilis* on tumoral cells and *Trypanosoma* parasites. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 112C(2):119–128

Mullin ChA, Gonzalez-Coloma A, Gutierrez C, Reina M, Eichenseer H, Hollister B, Chyb S (1997) Antifeedant effects of some novel terpenoids on chrysomelidae Beetles: comparisons with alkaloids on an alkaloid-adapted and nonadapted species. J Chem Ecol 23:1851–1866

Muñoz O, Montes M, Wilkomirsky T (2001) Plantas medicinales de uso en Chile, química y farmacología. Editorial Universitaria. Universidad de Chile. Vicerrectoría de Asuntos Académicos. Santiago, Chile

Muñoz E, Lamilla C, Marin JC, Alarcon J, Cespedes CL (2013a) Antifeedant, insect growth regulatory and insecticidal effects of *Calceolaria talcana* (*Calceolariaceae*) on *Drosophila melanogaster* and *Spodoptera frugiperda*. Ind Crop Prod 42:137–144

Muñoz E, Escalona D, Salazar JR, Alarcon J, Cespedes CL (2013b) Insect growth regulatory effects by diterpenes

from *Calceolaria talcana* Grau & Ehrhart (Calceolariaceae: Scrophulariaceae) against *Spodoptera frugiperda* and *Drosophila melanogaster*. Ind Crop Prod 45:283–292

Muñoz E, Avila JG, Alarcón J, Kubo I, Cespedes CL (2013c) Tyrosinase inhibitors from *Calceolaria integrifolia* s.l.: *Calceolaria talcana* aerial parts. J Agric Food Chem 61:4336–4343

Muñoz MA, Chamy C, Bucio MA, Hernandez-Barragan A, Joseph-Nathan P (2014) Absolute configuration of scopadulane diterpenes from *Calceolaria* species. Tetrahed Lett 55:4274–4277

Nicoletti M, Galeffi C, Messana I, Garbarino JA, Gambaro V, Nyandat E, Marini-Bettolo GB (1986) New phenylpropanoid glucosides from *Calceolaria hypericina*. Gazz Chim Ital 116(8):431–433

Nicoletti M, Galeffi C, Messana I, Marini-Bettolo GB, Garbarino JA, Gambaro V (1988a) Studies in *Calceolaria* genus. Part 2. Phenylpropanoid glycosides from *Calceolaria hypericina*. Phytochemistry 27(2):639–641

Nicoletti M, Galeffi C, Multari G, Garbarino JA, Gambaro V (1988b) Studies on the genus *Calceolaria*. Part 3. Polar constituents of *Calceolaria ascendens*. Planta Med 54(4):347–348

Ortego F, Rodriguez B, Castañera P (1995) Effects of *neo*-clerodane diterpenes from *Teucrium* on feeding behavior of Colorado potato beetle larvae. J Chem Ecol 21:1375–1386

Ortego F, López-Olguín J, Ruíz M, Castanera P (1999) Effects of toxic and deterrent terpenoids on digestive protease and detoxication enzyme activities of Colorado potato beetle larvae. Pestic Biochem Physiol 63:76–84

Pang YP, Brimijoin S, Ragsdale DW, Zhu KY, Suranyi R (2012) Novel and viable acetyl- cholinesterase target site for developing effective and environmentally safe insecticides. Curr Drug Targets 13:471–482

Passi S, Nazzaro-Porro M (1981) Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds. Brit J Dermatol 104(6):659–665

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. Recent Adv Phytochemistry 10:168–213

Rhodes SHL, Fitzmaurice AG, Cockburn M, Bronstein JM, Sinsheimer JS, Ritz B (2013) Pesticides that inhibit the ubiquitin-proteasome system: effect measure modification by genetic variation in SKP1 in Parkinson's disease. Environ Res 126:1–8

Rosner D, Markowitz G (2013) Persistent pollutants: a brief history of the discovery of the widespread toxicity of chlorinated hydrocarbons. Environ Res 120:126–133

Ryan JD (1979) Proteinase inhibitors. In: Rosenthal GA, Janzen DH (eds) Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, pp 599–618

Sachetti G, Romagnoli C, Nicoletti M, Di Fabio A, Bruni A, Poli F (1999) Glandular trichomes of *Calceolaria adscendens* Lidl. (Scrophulariaceae): histochemistry, development and ultrastructure. Ann Bot 83:87–92

Serrano A, Palacios C, Roy G, Cespon C, Villar ML, Nocito M, Gonzalez-Porque P (1998) Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys 350:49–54

Simmonds MSJ, Blaney WM, Esquivel B, Rodriguez-Hahn L (1996) Effect of clerodane-type diterpenoids isolated from *Salvia* spp on the feeding behaviour of *Spodoptera littoralis*. Pestic Sci 47:17–23

Simmonds MSJ, Manlove JD, Blaney WM, Khambay BPS (2002) Effects of selected botanical insecticides on the behaviour and mortality of the glasshouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*. Entomol Exp Appl 102:39–47

Singh ShB, Graham PL, Reamer RA, Cordingley MG (2001) Discovery, total synthesis, HRV 3C-protease inhibitory activity, and structure-activity relationships of 2-methoxystypandrone and its analogues. Bioorg Med Chem Lett 11:3143–3146

Singh-Ratan R (2010) Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot 29: 913–920

Swain T (1979) Tannins and lignins. In: Rosenthal GA, Janzen DH (eds) Herbivores: their interactions with secondary plant metabolites. Academic Press, New York, pp 657–682

Takemoto K, Kamisuki Sh, Chia PTh, Kuriyama I, Mizushina Y, Sugawara F (2014) Bioactive Dihydronaphthoquinone Derivatives from *Fusarium solani*. J Nat Prod. doi:10.1021/np500175j

Tene V, Malagón O, Finzi PV, Vidari G, Armijos C, Zaragoza T (2007) An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J Ethnopharmacol 111(1):63–81

Thomas E, Vandebroek I, Sanca S, Van Damme P (2009) Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. J Ethnopharmacol 122(1):60–67

Torres P, Ávila JG, Romo de Vivar A, García AM, Marín JC, Aranda E, Cespedes CL (2003) Antioxidant and insect growth regulatory activities of stilbenes and extracts from *Yucca periculosa*. Phytochemistry 64:463–473

Truman JW, Riddiford LM (2002) Endocrine insights into the evolution of the metamorphosis in insects. Annual Rev Entomol 47:467–500

Valladares G, Defago MT, Palacios SM, Carpinella MC (1997) Laboratory evaluationof Melia azedarach (Meliaceae) extracts against the elm leaf beetle (Coleoptera:Chrysomelidae). J Econ Entomol 90:747–750

Woldemichael GM, Waechter G, Singh MP, Maiese WM, Timmermann BN (2003) Antibacterial diterpenes from *Calceolaria pinifolia*. J Nat Prod 66(2):242–246

Wollenweber E, Mann K, Roitman JN (1989) Flavonoid aglycons excreted by three *Calceolaria* species. Phytochemistry 28(8):2213–2214

Xie Y, Isman MB, Feng Y, Wong A (1993) Diterpene resin acids: major active principles in tall oil against variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae). J Chem Ecol 19:1075–1084

Yamane H, Konno K, Sabelis M, Takabayashi J, Sassa T, Oikawa H (2010) Chemical defence and toxins of plants. In: Mander L, Lui H-W (eds) Comprehensive natural products ii chemistry and biology, vol 4. Elsevier, Oxford, pp 339–385

Zhu F, Poelman EH, Dicke M (2014) Insect herbivore-associated organisms affect plant responses to herbivory. New Phytol. doi:10.1111/nph.12886