Chapter

Modulation of Secondary Metabolites among Mexican Medicinal Plants by Using Elicitors and Biotechnology Techniques

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Abstract

Medicinal plants are being utilized as raw material and the use has increased in recent decades due that these biosynthesize compounds with several pharmacological activities. Some plant species with biological potential are of interest to the industry for preparation of drugs, phytodrugs, or food supplements. This causes overexploitation and deforestation, which endangers plant species-of-interest. In recent years, alternatives have been sought to eradicate this problem. A solution that was give and is maintained is plant biotechnology, which favors the production of active Secondary Metabolites (SMt). Plant biotechnology allows us to increase the yield of a compound-of-interest, reduces its production times and costs, and allows constant and controlled production of the raw material, and while aiding in the protection of medicinal plants that are found in danger of extinction. In the scientific literature, procuring the SMt by means of biotechnological processes is described, highlighting the study of four species from Mexican traditional medicine (*Lopezia racemosa*, *Galphimia glauca, Cnidoscolus chayamansa, Sphaeralceae angustifolia* and *Buddleja cordata*), and the main biological activities are as follows: anti-inflammatory, hepatoprotector, neuroprotector, anxiolytic, antitumoral, antibacterial, and antioxidant, among others.

Keywords: Elicitors, biotechnology, Mexican medicinal plants, plant tissue culture, secondary metabolites, phenolic compounds

1. Introduction

Ever since our ancestors, humanity has been dependent on the consumption of plants as a source of food, health, and for construction/ornamental. In addition, plants have developed a complex defense system against biotic and antibiotic stress: therefore, they can produce diverse secondary metabolites (SMt). The stress to which plants are submitted under natural conditions is caused by different factors, among

which stand out: attack by diverse insects and/or microorganisms (viruses, bacteria, and fungi) competition for soil, light, and nutrients, and exposure to sunlight [1].

SMt are compounds that do not play a fundamental role in the vital processes of plants, but they are important as mechanisms of defense. They are responsible for organoleptic and protective properties, such as odor, flavor, color, and consistency. These SMt also act as chemoattractants or chemorepellents. In addition, they are of great interest in industry for the preparation of food additives, agrochemicals, essences, biodiesel, narcotics, insecticides, cosmetics, and aromatics, and one of the most important of these is for the production of substances with pharmaceutical interest. Frequently, the production of SMt wild-collected plant is very low (less than 1% of the plant's Dry Weight -DW-), and this depends specifically on the plant's physiological state, the geographic location, the climate, among other factors [2].

Due to the low yield of SMt in wild plants and considering its important biological activity, alternatives or tools are currently being sought to increase its yield. One of these alternatives is the application of several biotechnology processes, a discipline that is oriented toward the development and innovation of technologies that involve the management of biological material for the production of a good or service [3].

One of the advantages in the use of biotechnological processes is to increase the production of bioactive SMt and also reduce the production time, which favors their availability [4]. The purpose of this paper is to summarize all the information that exists on the use of biotechnological processes for the production of bioactive compounds from Mexican medicinal plants.

2. Products with pharmaceutical importance

Plants constitute a huge reservoir of chemical structures, the most economically important are medicinal plants, due to their diverse biological activities; which over the years have favored human survival thanks to their use in Traditional Medicine (TM) [5–7]. TM is widely used in some developing countries, where their health system is still growing and is of great economic importance. In Africa, up to 80% of the population employs TM to help satisfy its health needs. In Asia and in Latin America, the populations continue to use TM because of historical circumstances and cultural beliefs. In China, TM is of great importance due to the large percentage of population that utilizes it, being higher than 60%. In some developed countries, the percentage of the population that uses TM is 48% in Australia, 70% in Canada, 42% in the USA, 38% in Belgium and 75% in France [8].

Currently, Medicinal Plants (MP) are employed by 80% of the world population; therefore, these are overexploited not only because are source of active ingredients, also due to the high nutritional, wood, cosmetic, agricultural, and/or medicinal value that many of these have. For example, it is estimate that China exports 120,000 tons of MP and India, some 32,000 tons while Europe imports 400,000 tons of MP. This leads to overexploitation of the species and many of them are in danger of extinction [9, 10].

The World Conservation Union and the World Nature Fund report that there are between 350,000 and 550,000 species of MP in the world, of which only approximately 20% possess documented investigation of their biological potential, and nearly 15,000 species are in danger of extinction due to the overexploitation and destruction of habitats [10, 11].

Nowadays, scientific interest in MP has increased due to the high costs and adverse effects that allopathic drugs cause, in addition to the increasing appearance of strains of microorganisms that are resistant to current treatments [12–14]. It is noteworthy that almost 25% of the active principles of allopathic drugs currently used were isolated and/or semisynthesized from plants [9]. In modern medicine, digoxin is use as a cardiotonic and was isolated from *Digitalis purpurea* (purpura, its common name); escin is use as an anti-inflammatory and venotonic and was isolated from *Aesculus hippocastanum* (its common name, horse chestnut). Another compound utilized is ajmalicin, employed for circulatory disorders, and was isolated from *Rauwolfia serpentina*; paclitaxel (an anticancer drug) has been semisynthesized by Bristol-Myers Squibb since 2002, and was obtained from the compound 10-deacetylbacatin III, it was obtained from the cell suspension of the *Taxus baccata.* While diosgenin, a steroidal sapogenin, was obtained from the tubercules of several *Dioscorea* species, which was the raw material for the semisynthesis of progesterone [15].

Guanidine is a natural product with good hypoglycemic activity that was isolated from *Galega officinalis* (L); however, this compound has been reported to be toxic for human consumption. Therefore, this compound was semi-synthesized obtaining metformin (dimethylbiguanide), which is less toxic and has a pharmacologic effect similar to the original molecule and is widely used for the treatment of type II diabetes mellitus. It is worth noting, due to the high demand for SMt on the market; several companies have seen the need to discover novel sources of raw material from MP [16].

On the other hand, at present, the use of medicinal plants and/or phytodrugs is very frequent. The phytodrugs are elaborate with plant material and some derivatives of this. The main ingredient is the aerial or subterranean plant's part; as well as extracts, tinctures, juices, resins, fatty acids, and essential oils presented in pharmaceutical form. The therapeutic effectiveness and safety have been confirmed scientifically [17]. Some examples of these include ginseng, it is obtained from Panax genus (*Panax ginseng* and *P. quinquefolium*) native from Asia and America, respectively. The main biological effect of ginseng "tonic" phytodrug is that it possesses the ability to increase the capacity to tolerate tensions, which leads to increased mental and physical yield. Another phytodrug obtained from St. John's wort (*Hypericum perforatum*) is Hiperikan, which is standardized based on its content of hypericin; its principal pharmaceutical use is against depression. *Ginkgo biloba* (Ginkgo) belongs to the Ginkgoaceae family, the active compounds in the leaf's extracts are gingolides (gingolides A-C, J, and M), along with a mix of sesquiterpene lactones and flavonoids which is used against depression. The majority of commercial preparations from Ginkgo are standardize with approximately 5–7% of terpenic lactones and 22–27% of flavonoids and they are employed mainly for the treatment of the cognitive deterioration associated with alterations in blood circulation in the brain, such as dementia. The phytodrug elaborated with *Echinacea purpurea* is commercially known as EchinaCold (Schwabe Pharma) or Immulone (ATOS Pharma). These are standardized on based of the echinacosides (caffeic acid derivative) content, whose main biological effect is as an immunostimulant [18]. In Oceania region, the extract from *Piper methysticum* (from root and rhizome) has the commercial name Kava-kava (with 30% of kava lactones), and is utilized for their neurotransmitter activity [19]. Another phytodrug is Vitango, obtained from *Rhodiola rosea* (with 3.5% rosavins and 1% salidrosides), and it is employed for reducing the stress associated with physical and mental tasks [20]. Plantival has extract mixture from *Valeriana officinalis* (160 mg) and *Melissa officinalis* (80 mg) and is use in the treatment of nervousness, restlessness and insomnia as an anxiolytic and antidepressive [21]. Another phytodrug, known as

Prostasan, is the extract of *Serenoa repens*, standardized at 25% of fatty acids; the dose employed is 160 mg, and its principal effect is antiandrogenic and against benign prostatic hyperplasia [22].

Due to the acceptance and growing use of phytodrugs around the world, PM are raw materials of great attention due to high consumption. In addition, MP biosynthesize several bioactive compounds, which are classified as terpenoids, alkaloids, lactones, flavonoids, coumarin, lignans and phenols, among others; many of these have restrictive taxonomical distribution. Although the SMt functions are not directly associated with the plant's basic function, these compounds carry out some interaction roles in the plant and its environments such as: protection against pathogens, protection against abiotic tensions (ultraviolet radiation radiation), they possess the function of attracting pollinating insects, and they are signaling molecules and active ingredients for drugs [23–25].

It is estimated that around 50% of the drugs approved by the Federal Drug Administration are products derived from natural sources or analogs deriving from plants or microorganisms [26]. However, raw material can be limited, and its exploitation is one of the main ecological concerns. One of the key objectives of plant biotechnology is the development of large-scale production methods of pharmacologically active products. Additionally, the massive biosynthetic potential of plants has not been completely exploited yet and biotechnology can be employed to generate new chemical compounds that possess unknown biological activities and/or with a different mechanism of action, or a better one, than those in existence [23].

3. Production of SMt by biotechnology tools

There are distinct strategies to optimize the production and modulation SMt in medicinal plants and food. The main strategies are by uses the elicitors (molecules capable of inducing defense in the plant) [1], which are classified as biotic and abiotic. Biotics are of biological origin, while abiotics can be physical or chemical. Some examples of physical abiotics are the weather, bacteria, and plagues, among others, while chemical abiotics possess an intense variety, with those most utilized being jasmonic acid and salicylic acid [27, 28]. One of the advantages of using elicitors treatment is that they function as signaling compounds for the mechanisms of defense; thus, they increase the production of SMt in an effective and rapid manner [29]. There is great specify in the interaction of plant-elicitor species which implies that the adequate one for each culture, the time of adding it, and the concentration for obtaining best response should be selected [30].

There is other technique very used to obtain SMt *in vitro*, it focuses on obtaining the roots, which is known as "hairy roots" or transformed roots; for this, the bacterium *Agrobacterium rhizogenes* is very used. This microorganism transfers the plasmid of the Transfer-DNA (T-DNA) of the T-DNA to the plant cell, to verify whether a root transformation was obtained, this can be confirmed by Southern hybridation analysis (this technique permits the detection of a specific DNA sequence in a complex mixture). A main advantage of these is that they have the capacity of rapid growth without the external administration of Plant Growth Regulators (PGR); the majority of these do not require a light supply, and their yield of metabolites is constant due to their genetic stability [1]. Another internal factor is the culture medium added with macro- and micronutrients, as well the external factors, such as light intensity, temperature, humidity, and stirring speed [31].

In general, formulation of the culture medium begins with the base medium, being the most utilized Muashige & Skoog (MS), B5 of Gamborg and Linsmaier and Skoog (LS), and Nitsch and Nitsch (NN) [32]. These culture mediums contain minerals, vitamins, and a carbon source, normally sucrose and sometimes fructose is used. Although plant cell cultures typically are initiate in solid medium, they require liquid medium for production on a large scale. The mineral content and/or the carbon source in culture medium have a profound impact on biosynthesis of SMt employed in the manufacturing of phytodrugs and/or compound-of-pharmaceutical-interest [33].

Other tools very used to obtain SMt by biotechnological process is through the use of BioCatalyzers; this method has been used to transform polyphenols compounds; for example, *Bouvardia ternifolia* is utilized for the production of a BioC denominated dehydrodiisoeugenol, which was obtained from the supernatant of cells suspension, demonstrating a yield of around 77%. The dehydrodiisoeugenol obtained from *B. ternifolia* allows the production of isoeugenol by biotransformation; it is known that plant peroxides transform phenols substituted for by a methyl group orto position to the corresponding O-radical, which, on establishing itself by resonance, produces a C-radical; the latter is that which leads to dimerization, producing a dimer. This biotransformation represents a clean and green alternative with respect to traditional chemical methods, in which oxidative bonding reactions are affected using catalysts such as $FeCl_3$, $K_3(FeCN)_6$, and Cu(OH)Cl [34].

Recently, interest in research and development of *in vitro* plant tissue cultures from MP has grown; however, there are scarce studies, to our knowledge, in which the biological activities of these SMt obtained by this process are described. The majority of works published only mention the conditions of the biotechnological process and the final concentrations of the different metabolites produced, but do not evaluate the pharmacological activity of these SMt, and the authors solely cite that these have been reported in previous works.

In **Table 1** and **Figure 1**, some examples are described. It is important to mention that on some occasions is difficult to establish the biotechnological process conditions to induce the biosynthesis of bioactive SMt from a MP.

Species	Products	Use	References
Alanthus altissima	Alkaloids	Antimicrobial	[35]
Ajuga reptans	Antocyanins	Antioxidant	[36, 37]
Alanthus altissima	Cantinones alkaloids	Antimicrobial	[38]
Ammi majus	Coumarins	Anticoagulant	[39]
Anchusa officinalis	Rosmarinic acid	Antioxidant	[40, 41]
Anthoceros agrestis	Rosmarinic acid and Glycosides	Antioxidant	[42]
Arachis hypogea	Piceatannol	Antioxidant	[43]
Artemisia annua	Artemisinin	Antimalaric	[30, 44]
Artemisia judaica	Flavonoids	Antioxidant	[45]
Bouvardia ternifolia	Dehydrodiisoeugenol	Biocatalyst	[34]
Beta vulgaris	Betalains	Antioxidant	[46, 47]
Buddleja cordata	Verbascoside (**1**), linarin (**2**)	Anti-inflammatory, antioxidant	[48]

Species	Products	Use	References
Buddleja cordata	Phenylpropanoids	Antioxidant	[49]
Caesalpinia pulcherrima	Homo isoflavones	Antimicrobial, antitumoral	[50]
Calophyllum inophyllum	Dipyranocoumarins	AntiHIV	[51]
Camelia sinensis	Thiamine or theanine	Antihypertensive	[52]
Capsicum frutescens	Capsaicin (**3**)	Irritant	[53]
Cassia acutifolia	Antraquinones	Antimicrobial	[54]
Castilleja tenuiflora	Verbascoside (**1**), Isoverbascoside, aucubin	Anti-inflammatory, Antispasmodics	[55]
Catharanthus roseus	Ajmalicin	Antihypertensive	[56]
Catharanthus roseus	Vinblastin	Anticancer	[57]
Cecropia obustifolia *Cecropia peltata*	Chlorogenic acid, Isoorientin	Hypoglycemic	[58]
Cephaelis ipecacuana	Emetin	Antiparasitic	[59]
Cephalo-taxus fortunei	Abietane diterpenoids	Antitumoral	[60]
Choisya ternata	Furanocoumarins	Antitumoral, Antioxidant	[61]
Choisya ternata	Furoquinolin alkaloids	Antitumoral, Antimicrobial	[62]
Cinchona robusta	Robustaquiones	Antimalarial	[63]
Cistanche deserticola	Glycosides	Antioxidant	[64]
Cistanche salsa	Glucophenyletanoids	Aphrodisiac	[65]
Colchium autumnale	Colchicine (**4**)	Antitumoral	[66]
Coleus forskolii	Forskolin (**5**)	Asthma	[67]
Comptotheca acuminate	Camptotecin (**6**)	Antitumoral	[68]
Coptis japonica	Berberin (**7**)	Intestinal infection	[69]
Cornus kousa	β-glucogallin, (+)-Catechin, (+)-gallocatechin, procyanidin B-3	Hyperglycemic and antimicrobial	[70]
Coscinium fenestratum	Berberin	Antioxidant, Antidiabetic	[71]
Crocus sativus	Crocin	Anticancer	[72]
Cynara cardunculus	Cinarin, Chlorogenic acid	Antioxidant	[73]
Daucus corata	Antocyanins	Lipoperoxidation	[74]
Digitalis lanata	Digoxin (**8**)	Cardiostimulant	[75]
Dioscorea deltoide	Diosgenin (**9**)	Steroidal stimulant	[76]
Drosophyllum lusitanicum	Plumbagin (**10**)	Anticancer, Antimicrobial	[77]
Eleutherococcus sessiliflorus	Eleuteroside	Anti-inflammatory, diuretic, analgesic, antipyretic	[78]

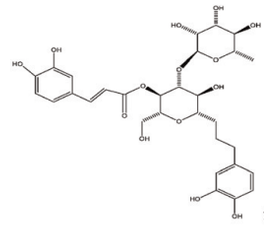
Species	Products	Use	References
Eriobotrya japonica	Triterpenes	Anti-inflammatory, antidiabetic, antitumoral	[79]
Eucommia ulmoides	Chlorogenic acid (**11**)	Antimicrobial, Antioxidant	[80]
Fagopyrum esculentum	Rutin (**12**)	Antioxidant	[81]
Fragaria ananassa	Antocyanins	Antioxidant	[82]
Galphimia glauca	Galphimine B (**27**)	Central nervous system disorders	[83]
Glehnia littoralis	Antocyanins	Antioxidant	[84, 85]
Gymnema sylvestre	Gymnemicanor	Antidiabetic	[86]
Helianthus tuberosus	Inulin	Antidiabetic	[87]
Hemidesmus indicus	Rutin (**12**)	Antioxidant	[88]
Hypericum perforatum	Hypericin	Antidepressive	[89]
Hyssopus officinalis	Rosmarinic acid (**13**)	Antioxidant	[90]
Hyssopus officinalis	Lithospermic acid	Antioxidant	[91]
Ipomoea batatas	Antocyanins	Antioxidant	[92–94]
Larrea divaricata	Nordihydroguayaretic acid, Quercetin	Antiarthritic, digestive, against venereal diseases	[95]
Lavandula vera	Rosmarinic acid (**13**)	Hepatoprotective	[96, 97]
Leucophyllun frutescens	Coumarins, lactones, flavonids	Antioxidants	[98]
Lithospermum erythrorhizon	Shikonin	Antibacterial	[99]
Lopezia racemosa	6-O-palmitoyl-3-O-β-D-glucopyranosylcampesterol, 6-O-palmi-toyl-3-O-β-D-gluco-pyranosyl-β-sitosterol	Anti-inflammatory	[100]
Lycium chinense	Cerebroside	Cellular Growth Regulator	[101]
Morinda elliptica	Antraquinones	Antimicrobial	[102]
Mucuna pruriens	L-dihydroxyphenylalanine	AntiParkinson	[103]
Ochrosia elliptica	Elipticin	Antitumoral	[104]
Ocimum basilium	Rosmarinic acid (**13**)	Antioxidant	[105]
Panax ginseng	Ginkgolides (**14**)	Cognitive deterioration	[106]
Panax ginseng	Ginsenosides (**15**)	Immunomodulator	[107]
Papaver somniferum	Codeine (**16**)	Sedative	[108]
Papaver somniferum	Morphine (**17**)	Sedative	[109]
Papaver somniferum	Sanguinarin (**18**)	Platelet stimulator	[110]
Passiflora quadrangularis	Orientin, Isoorientin, Vitexin, Isovitexin	Antioxidant	[111]
Petroselinum sativum	Flavonolides	Antioxidant	[112]

Species	Products	Use	References
Picrasma quassioides	Cuasin	Antiphylogistic	[113]
Piqueria trinerva	Monoterpene	Antifungal	[114]
Podophyllum hexandrum	Podophylotoxin (**19**)	Antitumoral	[115]
Psoralea corylifolia	Genistein and Daidzein	Tonic	[116]
Rauwolfia serpentina	Reserpin (**20**)	Antihypertensive	[117]
Rubia tinctorum	Antraquin-ones	Antimicrobial	[118]
Salvia miltiorrhiza	Cryptotanshinone	Antioxidant, antimicrobial	[119]
Salvia miltiorrhiza	Tanshinone	Cardiac problems	[120]
Saussurea medusa	Hispiduline (**21**), Jaceosidine	Antitumoral	[121]
Silybum marianum	Silymarin	Hepatoprotective	[122]
Solanum malacoxylon	Cholecalcipherol	Aids calcium absorption	[123]
Sphaeralcea angustifolia	Sphaeralcic acid (**22**), Tomentin (**23**)	Antiinflammatory	[124]
Swietenia humilis Zucc.	Alkaloids	Cytotoxic	[125]
Tanacetum parthenium	Parthenolide (**24**)	Anticancer	[126]
Taxus brevifolia	Taxol (**25**)	Anticancer	[127]
Vitis vinífera	Antocyanins	Antioxidant	[128]
Vitis vinífera	Resveratrol (**26**)	Antioxidant, Hepatoprotective	[129]
Withania somnifera	Withanolide A	Antioxidant, Antistress	[130]

Table 1.
Secondary metabolites obtained for cellular cultures from medicinal plant tissues in vitro *and their biological activity.*

4. Anti-inflammatory activity of SMt isolated from vegetal material obtained by biotechnological processes

Some SMt with significant anti-inflammatory activity have been obtain from MP through employment some biotechnological processes. From cell suspension cultures *Sphaeralcea angustifolia*, two compounds with important anti-inflammatory activity (evaluated in murine models) were isolated. The cell suspension was developed in MS medium with total nitrate 2.74 mM, under this condition was obtained scopoletin, sphaeralcic acid (**22**) and tomentin (**23**). From the CH_2Cl_2:CH_3OH extract sphaeralcic acid (**22**) and tomentin (**23**) were isolated; these compounds showed 58 and 66% anti-inflammatory activity, in the carrageenin model at 45 mg/kg administered by intraperitoneal (i.p.) route. On the other hand, in the topical anti-inflammatory model (TPA, 12-O-TetradecanoylPhorbol-13-Acetate), tomentin (225 mM/ear) exhibited 57% inhibition in the formation of auricular edema, while sphaeralcic acid (174 mM/ear) revealed 86% inhibition with a dose-dependent effect and one half of the Effective Dose (ED_{50}) = 93 mM. Sphaeralcic acid is the most active compound in both models (topical as well as systemic) [124, 131].



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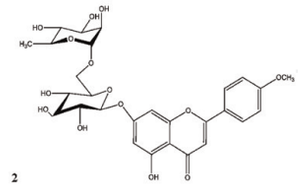

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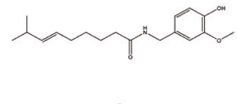

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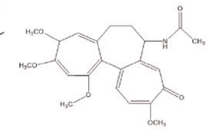

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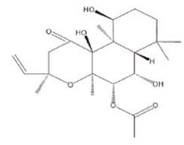

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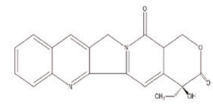

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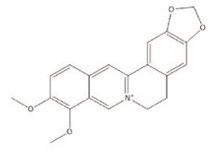

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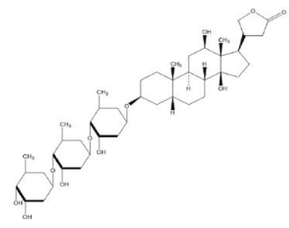

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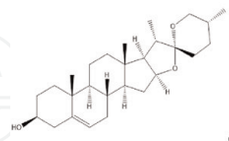

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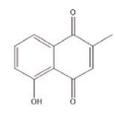

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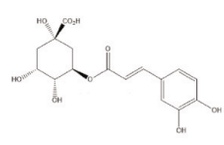

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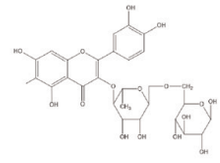

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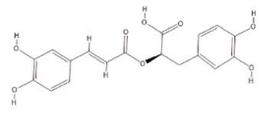

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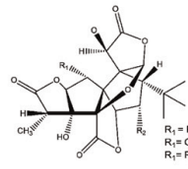

$R_1 = R_2 = H$, ginkgolide A
$R_1 = OH$, $R_2 = H$, ginkgolide B
$R_1 = R_2 = OH$, ginkgolide C

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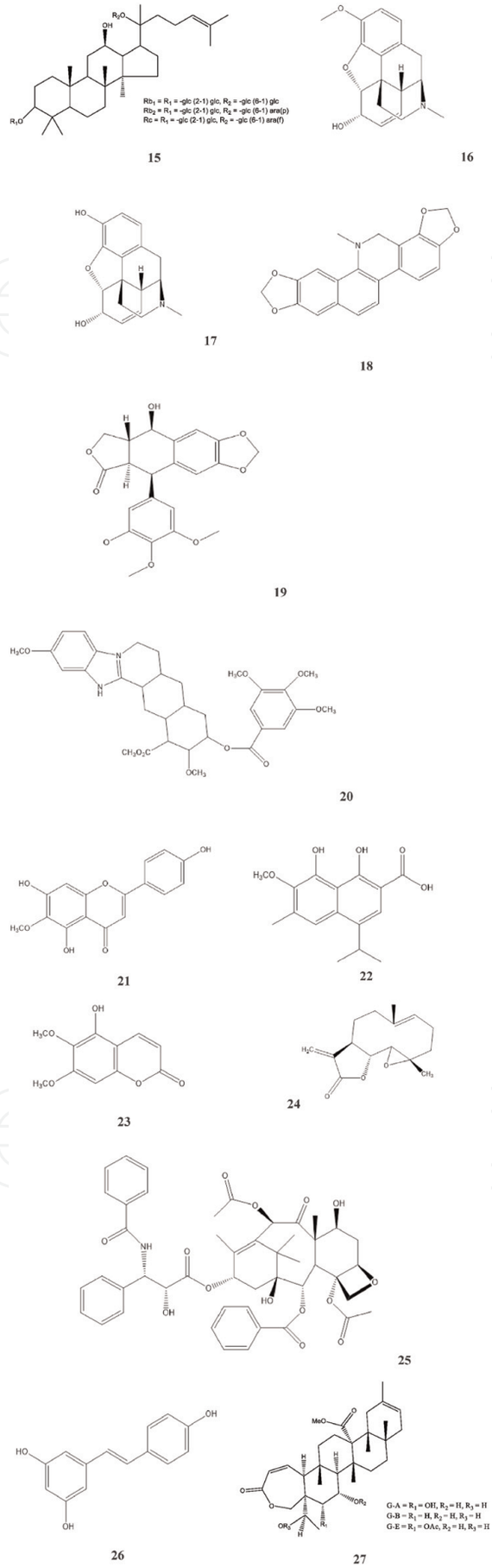

Figure 1.
Chemical structure of some polyphenols and other SMt with biological activity.

In another study, the anti-inflammatory activity of the cell suspension culture from *S. angustifolia* is described. In this case, aseptic-leaf explants and Naphthalene Acetic Acid (NAA, such as auxin) in several concentrations (0, 0.5, 1.0, and 2.0 mg/L) in combination with a constant concentration of Kinetin (KIN) were used. For the cell suspension culture, they utilized 4% initial inoculum in MS medium with 2.74 mM of the total nitrates, 1 mg/L of NAA and 0.1 mg/L of KIN and supplemented with 30 g/L of sucrose. The main SMt identified in this suspension cultures were the same compounds (scopoletin, tomentin, and sphaeralcic acid). Scopoletin was excrete in the culture medium, although it also accumulated in the biomass. For evaluation of the anti-inflammatory activity, the authors prepared the CH_2Cl_2:MeOH extract of the cell's suspension from *S. angustifolia* and this extract was administered i.p. in male ICR mice (35 g) employing the carrageenin model. This extract showed ED_{50} = 137.63 mg/kg; sphaeralcic aid and tomentin at 45 mg/kg inhibited 67 and 62%, respectively on carrageenan assay and sphaeralcic acid at 1 mg/ear was more active in TPA assay, showed ED_{50} = 93 mM and tomentin showed 48% of inhibition at 1 mg/ear [132, 133]. In addition, the same extract from biomass of cells in suspension of *S. angustifolia* at 100 mg/kg (with 0.10 mg of scopoletin, 0.10 mg of tomentin and 0.19 mg of sphaerelcic acid), as well as tomentin (20 mg/kg) were active as anti-inflammatory agent and reduced the mean body weight lost in Freund adjuvant- and kaolin/carrageenan-induced arthritis, respectively. In this assay, the organic extract and tomentin reduced the levels of pro-inflammatory interleukins such as IL-1β, IL-6 and TNF-α and increased levels of IL-4 and IL-10 (anti-inflammatory cytokines) [133].

In parallel with obtaining cells *in vitro* of *S. angustifolia*, the authors performed a preclinical phase study (in rats). The CH_2Cl_2 extract of the aerial parts of *S. angustifolia* (wild material) was tested in chronic inflammation model induced with complete Freund's adjuvant (polyarthritis) The administration of the extract at 100 mg/kg/day during 8 days showed sustained and significant inhibition of edema, being of 62.6% [134]. A double-blind clinical phase study with the extract of *S. angustifolia* (wild material) standardized at 1% hydroxycoumarin content was conducted; the experiment was performed on 130 patients diagnosed with osteoarthritis. 55 of them were treated with standardized extract of *S. angustifolia* (gel) and 75 patients were treated with Diclofenac (2%). The therapeutic effectiveness of the gel administered topically for 4 weeks was 89%, while that of the control group (Diclofenac) was 91.3%; it was highlighted in the study that patients who received the treatment (gel of the standardized extract) did not exhibit adverse effects and did show an improvement in their disorder [135].

Another plant utilized in Mexican ethnomedicine is *Lopezia racemosa* Cav. Callus cultures in MS medium were obtain with variable amounts of NAA, 2,4-Dichlorophenoxiacetic acid (2,4-D) and 6-BenzylAminoPurine (BAP). The authors carried out 10 treatments with the previously mentioned PGR. In this case, they employed three types of explants (hypocotyl, stem nodule, and leaf) and several treatments. The combination of 1.0 mg/L of 2,4-D plus 0.5 mg/L of BAP was the best.
From these callus material two novel compounds: 6-O-palmitoyl-3-O-β-D-glucopyranosylcampesterol (174.0 μg/g of biomass) and 6-O-palmitoyl-3-O-β-D-glucopyranosyl-β-sitosterol were isolated. When quantifying these compounds, the authors observed that the wild plant contains less quantity than the callus. The topical anti-inflammatory activity of the biomass obtained from the callus was evaluate in the TPA model on CD-1 male mice at 1 mg/ear. Three extracts (hexanic, CH_2Cl_2, and methanol), was tested and showed 48.74, 57.14, and 16. 81% of inhibition, respectively. The CH_2Cl_2 extract was the most active, with a half-maximal Inhibitory

Concentration (IC_{50}) = 0.93 mg/ear. On the other hand, the pure compound (6-O-palmitoyl-3-O-β-D-glucopyranosyl-campesterol) was tested in the same model at 1 mg/ear showing a 57.14% inhibition, with IC_{50} = 0.45 mg/ear [100].

The lipophilic extract containing beta-carotene (LMBC) from plant cell cultures of *Cleome spinosa* was evaluate in two *in vivo* models to determine the anti-inflammatory and antinociceptive activities in Swiss Webster (SW) mice of both sexes. The callus culture was obtained of the MS medium supplemented with 1 mg/L of 4- amino-3,5,6-trichloropicolinic acid (picloram) and sub-cultured to culture medium with the same composition at 4-week intervals. The anti-inflammatory activity in carrageenan model at 10 mg/kg by i.p. via was evaluated. LMBC was inactive with respect to extract from whole plant, which showed more than 50% inhibition of edema at the same dose. On the other hand, the LMBC (at 50 mg/kg) showed around 68% decrease in writhes, these data were very similar to that shown in wild plant, and the effect was better than dipyrone (at 100 mg/kg) used as positive control. The authors concluded that the results of LMBC are particularly important; since this active SMt of medicinal interest can be continuously obtain from callus cultures [136].

Buddleja cordata is other medicinal species utilized to treat diseases related with inflammation. This cell suspension was obtained in MS medium supplemented with NAA (9.05 μM) and Kin (2.32 μM). The anti-inflammatory activity of the extracts from wild plant and of the cell suspension cultures were describe. In both extracts, the verbascoside content was quantified by HPLC methods. The extract of the cells suspension has 87.48 mg verbascoside/g Dry Matter (DM), while that the same extract from wild plant only contained 47.34 mg of verbascoside/g DM. In addition, acute toxicity in Balb/C mice of the both extracts were also determined, with half of a Lethal Dose (LD_{50}) of >2 g/kg. On the other hand, the topical anti-inflammatory effect of the wild plant extract and of the cell suspension was assay. The ED_{50} values was 3.93 and 1.26 mg/ear, respectively, cell suspension extract was the most active due to its greater content of verbascoside. Evaluation of both extracts in the carrageenan model (systemic inflammation), showed ED_{50} = 251.26 and 204.62 mg/kg for wild plant and cells suspension extracts, respectively; in this case, the latter extract was more active. In the chronic inflammation model (the arthritis model induced with complete Freud's adjuvant), both extracts showed moderate anti-inflammatory activity (<35%) and favored weight increase in animals with arthritis. The authors concluded that the cell suspension culture of *Buddleja cordata* obtained through the biotechnological process contained a better anti-inflammatory activity; therefore, it represents a source for obtaining this type of secondary metabolite-of-pharmacological-interest [48].

Cnidoscolus chayamansa is medicinal plant whit anti-inflammatory, antiprotozoal, hepatoprotective, hypoglycemic and antimycobacterial activities [137–139]. Recently, a biotechnology processes was described to obtain callus using BAP (5 mg/L) and 2,4-D (2.5 or 5 mg/L), this callus was used as a biotechnological alternative for *in vitro* propagation of this plant [140]. After that, this callus was use to establish a cell suspension culture. From the cell suspension, organic extract was prepared and its antioxidant, antibacterial and anti-inflammatory activities were determined, as well as the main SMt was quantified by HPLC analysis. In cell suspension, lupeol acetate (38.1 mg/g DW) was obtained as a main constituent and scopoletin (3.6 mg/g DW) was also quantified; in wild material, both compounds were isolated in low quantity. The organic extract was active against *Staphylococcus aureus, S. coagulase* and *Listeria monocytogenes,* and a moderate antioxidant and anti-inflammatory activities (in TPA and carrageenan models) showed [28, 141].

5. Antineoplasic activity of the plant material obtained by biotechnological cultures

From the callus culture of *Eriobotrya japonica*, nine triterpenes (ursolic acid; oleanolic acid; maslinic acid; tormentic acid; 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid; 2α-hydroxyursolic acid; hyptadienic acid, and the mixture of 3-O-*cis*-p-coumaroyl tormentic acid and 3-O-*trans*-p-coumaroyl tormentic acid) were isolated. The main triterpenes of the callus tissues were tormentic acid (50 mg/g DW) and 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid (11.8 mg/g DW), the latter compound (2a,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid) is known as a potent protease inhibitor of the human immunodeficiency virus. All these triterpenes were tested in two cell lines (HSC-2 and HSC); seven of the nine triterpenes were active. Showing mean cytotoxic concentration (CC_{50}) between 10 and 48 μg/ml, while the oleanolic acid and 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid exhibited weak cytotoxic activity. Additionally, the authors evaluated the *in vivo* antitumor activity of the 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid in female ICR mouse skin (n = 15) during two stages of carcinogenesis; in this assay, carcinogenesis was induced topically with (+)-(E)-4-methyl 2[(E)-hydroxyimino]-5-Nitro-6-methoxy-3-hexenamide (NOR1) at a dose of 90 μg/0.1 mL of acetone. One week after NOR1 administration started, TPA (1 μg/0.1 mL of acetone) was administered twice weekly, yielding as a result a weak inhibition of the carcinogenesis. On the other hand, the authors mention that 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid is an antiproliferative agent and that the number of papillomas diminished by 40% in 20 weeks, indicating that this compound possesses potential for the delay of carcinogen in mouse skin [142].

6. Biological effect of SMt isolated from cell cultures of *Galphimia glauca*

Galphimia glauca is widely used in Mexican traditional medicine. From this species, some triterpenes such as Galphimine-A, B and E (**27**) have been isolated. These compounds showed a neuroprotective effect, when these were evaluated in mice convulsions model. To induce the seizure, the authors used strychnine or pentylenetratrazole administered by i.p. or subcutaneous route. In the study's results, the depressor effects observed on motor activity directed toward an objective or an aim [143]. The pharmacological effect of galphimine B (G-B) was due to selectively inhibiting the discharge of dopaminergic neurons in the central area in *in-vivo* models [144]. Due to its therapeutic importance of G-B, the authors proceeded to induce the production of this homogeneous raw material through a biotechnological process.

A first step was to obtain callus from hypocotyl explants in MS medium for 30 days with a combination of NAA and KIN; under these conditions, only great cell growth was obtained, and with 2,4-D at 4 mg/L the G-B production was stimulated with a yield of 0.154 mg/g DW. In addition, under this condition, G-E was also obtained but at less concentration (0.057 mg/g DW). Also, friable callus from suspension culture in MS medium with NAA and KIN (2:2 mg/L) was obtain, denominating this line as ggxl. By means of a growth kinetic, galphimines were shown to be produced in the culture's stationary stage [83, 145]. The next step was to carry out the scaling of galphimine production in the 5-liter *airlift* bioreactor and in one with mechanical stirring; the growth indices were 11.66 and 1.7, respectively. However, the authors observed that neither the biomass production, nor the time exerted an influence on the yield of G-B. Because the *airlift* produced a greater biomass but with lower yield of G-B (255 mg/L), while the stirring bioreactor at day 10 shown an intracellular as well as an extracellular

content of 1381 mg of G-B/L, 5.4-times higher than the *airlift* at day 25 [146]. Once the biotechnological conditions for the production of G-B were established, this allowed having raw material to carry out the pharmacological evaluation in different models.

7. Toxicologic effect of Galphimines

Aqueous extract from material obtained by bioreactor was prepared, whose galphamine content was G-A, G-B and G-E = 0.6, 1.034 mg/g, and 1.12 mg/g, respectively. Meanwhile, the content of these galphamine in the ethanolic extract was G-A = 5.35 mg/g, G-B = 18.8 mg/g, and G-E = 17.49 mg/g and the MeOH extract content G-A = 7.29 mg/g, G-B = 17.47 mg/g, and G-E = 11.6 mg/g. Afterward, each extract was administered to Balb/C male and female mice for 28 days (2.5 g/kg). During the study period, there were no deaths, and in the histopathological analysis of the different organs; the latter did not present alterations. Also, analyzed the behavioral parameters, demonstrating a reduction in spontaneous activity. Administration of these extracts for 56 days (2.5 g/kg) in mice did not cause any change in liver-function biochemical parameters. With regard to the cytotoxic evaluation in KB, UISO, and OVCAR-5 cell lines, no cytotoxic effects were found, but all of these extracts specifically inhibited growth of the colon-cancer cell line with ED_{50} of <2 μg/mL. On the genotoxicity test *in vitro*, the extracts were evaluated at three concentrations (250, 100, and 50 mg/mL) and none of the three *G. glauca* extracts showed a genotoxic effect [147].

8. Evaluation of the MeOH extract of *Galphimia glauca* in Behavioral models of anxiety

The anxiolytic and anti-depressive effects were evaluated for the *G. glauca* MeOH extract (wild material) standardized with content of G-B (8.3 mg/g), using the elevated light–dark labyrinth and forced swimming in albino (ICR) mice. The extract, administered orally, three times (24, 18, and 1 h prior to the test) at doses of 125, 250, 500, 1,000, and 2,000 mg/kg was capable of significantly increasing ($p < 0.05$) the number of entries, as well as time spent on the elevated labyrinth's open arms, which indicates an anti-anxiolytic effect. A similar effect was observed in the light–dark paradigm test: time spent in the light box increased in treated mice. However, this treatment was not able to change any parameter in the forced swimming test [148].

9. Conclusions

The MP form part of the daily life of the worldwide population. It is currently of scientific interest due to its high consumption, as an alternative treatment and/or co-administered with allopathic treatments for the improvement of chronic-degenerative diseases. On the other hand, the population has been responsible for affording a great boost to the use of MP; therefore, its consumption generates a great demand and consequently overexploitation. This overexploitation is a danger in the extinction of species of pharmaceutical interest. Another problem regarding the consumption of MP is that not all the population has access to species that are endemic and that have

great biological potential. All the above led to the search for methods to achieve the production and induction of SMt biosynthesis with important biological activity in less time, with constant, controlled and standardized production. Besides helping to preserve plant species without altering the ecosystem.

In some cases, has been reported that cell suspension cultures increase by up to 300% the production of SMt with biological interest respect to wild plant material. In addition, to the increase in SMt production, these are obtained in less complex mixtures, which facilitates the purification process. In the present work, we describe several SMt obtained for biotechnological processing; however, many of these SMt have not been submitted to *in vivo* studies that prove their potential biological activity. Therefore, it is necessary to develop projects aimed at obtaining metabolites by biotechnological processes and demonstrate their biological activity in *in vivo* models.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Nomenclature

SMt	Secondary Metabolites
SMBB	Sociedad Mexicana de Biotecnología y Bioingeniería
TM	Tradicional Medicine
MP	Medicinal Plants
DNA	Deoxyribonucleic acid
PGR	Plany Growth Regulators
BioC	BioCatalyzers
TPA	12-O-Tetradecanoyl Phorbol 13-Acetate
ED_{50}	Half of the Effective Dose
NAA	Naphthalene Acetic Acid
KIN	Kinetina
2,4-D	Dichlophenoxiacetic acid
BAP	6-BenzylAminoPurine

IC50	Hal-maximal Inhibitory concentration
DM	Dry Metter
LD_{50}	Half of a Lethal Dose
DW	Dry Metter
CC_{50}	Mean Cytotoxic Concentration
i.p.	Intraperitoneal
S.C.	Subcutaneous
G-A	Galphimine A
G-B	Galphimine B
G-E	Galphimine E
C.N	Kinetine

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