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Review Article

Cytochrome P450 from Plants: Platforms for Valuable Phytopharmaceuticals

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Abstract

Cytochrome P450 enzymes are important for biotechnology due to their capacity to modify diverse secondary metabolites that may produce chemicals with pharmacological properties. Most terpenes, flavonoids and alkaloids require P450 catalytic functions to reach their biological activity. In the last ten years, several efforts have focused on the expression and production of these three main types of secondary metabolites in engineered microorganisms and plants using P450 of ethnobotanical origin. Despite this, several P450 coding sequences from plant sources are discovered yearly but only a few have been screened by functional genomics. Amongst them, only a few have shown potentials for use in sustainable production of novel drugs and highly valuable products. Cytochrome P450 involvement in the biosynthesis of these products is discussed in this work.

Keywords: *Biotechnological platforms, Cytochrome P450, Phytopharmaceuticals, Yield improvement, Terpenes, Flavonoids, Alkaloids, Microbial expression*

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INTRODUCTION

Plant cytochrome P450 enzymes are important in biotechnology due to their ability to biosynthesize diverse secondary metabolites with biological properties. Numerous reports describe the application of P450 monoxygenases, desaturases and methyl transferases from plants as candidates for creating novel varieties of modified vegetables and bio-products [1-3]. P450 DNA coding sequences are also selected to originate pharmaceutical products, because of their importance as target genes for microorganism's metabolic engineering. Organic compounds with therapeutic uses and phytopharmaceuticals directly or indirectly derived from these enzymes are now recognized as critical alternatives for treating different illnesses. This is the case of taxol and the periwinkle alkaloids, which are widely used in cancer therapy [4].

Bioactive secondary metabolites are routinely extracted from the plant itself or produced by synthetic-chemistry in order to scale-up their production. Nevertheless, the sustainable production by those methods is not always successful [5]. Sometimes it is compromised

because of many features that distress plants like, the required phenological stage, the specific interactions with biotic and abiotic factors that modify their accumulation, as well as difficulties in the extraction procedures and all associated with high investments [6]. Due to these circumstances, many phytopharmaceuticals usually do not reach the clinical trials stage or the commercial attractive just decline. Currently, biotechnological platforms are gaining grounds in the sustainable production of pharmacologically active metabolites from plants. This alternative is not only in accordance with global ecological legislation, their implementation is also a promising and reliable tool for scaling plant metabolites production with complex chemical structures [7]. Despite the presence of other key enzymes actively involved in the biosynthesis of secondary metabolites, P450 are essential in almost all pathways of plant natural products. They are especially involved in the fine modification of the chemical skeletons originated by diverse synthases. In this review, we present relevant approaches in the production of promising pharmacological metabolites through molecular biotechnology using plant P450 enzymes in microorganisms and other plants.

GENETIC ENGINEERING OF P450 ENZYMES INVOLVED IN TERPENE BIOSYNTHESIS

Sesquiterpenes

Up to now, six sesquiterpenes have been successfully produced in heterologous systems by using P450 coding sequences (Figure 1). The anti-malarial drug, artemisinin (1) is a sesquiterpene lactone biosynthesized by *Artemisia* spp. It is used as a precursor of artemether which when combined with lumefantrine results in an exceptional drug against the tropical disease called Coartem ® by Novartis. As in many secondary metabolites, artemisinin is present in small quantities in the plant itself, thus limiting its extraction for therapeutic aims. Among first remarkable results on artemisinic acid production was by engineered *Saccharomyces cerevisiae* yielding over 100 mg L^{-1} [8].

A recent report described the optimization of artemisinic acid biosynthesis in *S. cerevisiae* by the insertion of distinct DNA coding sequences isolated from *A. annua* such as cytochrome b5 (*CYB5*), an artemisinic aldehyde dehydrogenase (*ALDH1*), an alcohol dehydrogenase (*ADH*) and the *CYP71AV1* genes [9]. The newly engineered yeast strains generated in this work were able to synthetize artemisinic acid in the order of 25 g L^{-1} when GAL promoters were induced to express the recombinant proteins. Therefore, artemisinic acid yields were increased ~250 times compared to the first attempt. The same report also describes an easy method for the chemical synthesis of artemisinin [9]. Orthologs of *CYP71AV1* gene were reported for several Artemisia species revealing polymorphisms that could influence the transformation of amorpha-4, 11-diene to artemisinic acid [10]. To date, engineered strains that actively biosynthesize artemisinic acid seem to be an attractive, cheaper and effective choice for industrializing the artemisinin production and also to decrease costs of the anti-malarial medicines in the short term.

Nootkatone is a sesquiterpene and the main natural and expensive substance of the smell and flavor of grapefruit. Nootkatone is also an effective and environmentally friendly repellent/insecticide against mosquitos, bed bugs, head lice and other insects [11]. It is non-toxic to humans and is an approved food additive commonly used in foods, cosmetics, and pharmaceuticals [11].

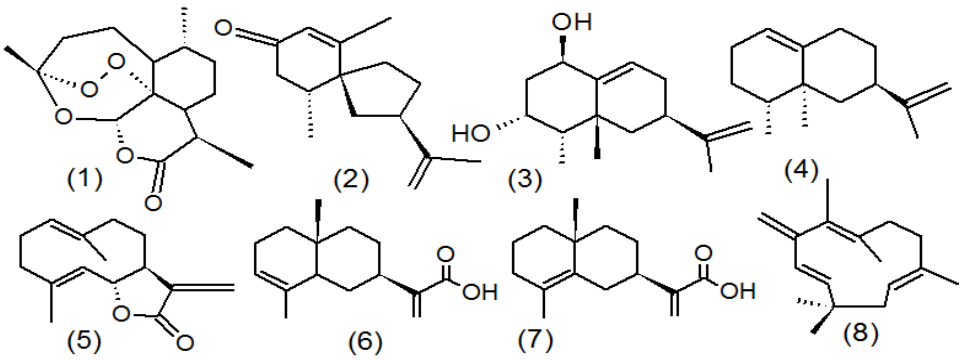

Figure 1: Some sesquiterpenes derived from P450 enzyme activity

Currently, flavor and fragrances are extracted from the oil of fruit peels to be used in drinks and perfumes. However and because supply of these fruits is limited and have a low amount of these substances, the price of nootkatone is around $ 2,000 a pound and the valencene (4) sells for $ 600 a pound or more [12]. Allylix (San Diego, CA) developed a fermentation process to produce nootkatone after engineering baker's yeast to produce a variety of smells and flavors [12].

The current knowledge of metabolic pathways of valencene and other sesquiterpenes like capsidiol (3), a bicyclic sesquiterpenic phytoalexin from *Capsicum* and *Nicotiana* plants [13]; is now used. The genes of 5-epi-aristolochene synthase and the valencene synthase enzymes (both with N-terminal thioredoxin modification) in combination with the 5-epi-aristolochene dihydroxylase (*CYP71D20*); all were inserted and differentially expressed in a specific yeast strain (*EPY300*). The transgenic organism was able to produce ~250 mg L-1 capsidiol and small quantities of valencene [14]. This improved the production of the phytoalexin in a heterologous system without the need of adding exogenous precursors.

Solavetivone (2) is an antifungal phytoalexin derived from a vetispirane-type sesquiterpene premnaspirodiene synthesized by *Hyoscyamus muticus*. Premnaspirodiene oxidase (HPO-*CYP71D55*) enzyme seems to be involved in the double oxidation of solavetivone and other vetispirane precursors, showing a multisusbtrate but very valuable activity [15].

Costunolide (5) is a germacranolide sesquiterpene lactone that shows potent anti-proliferative properties in different types of cancer [16]. Costunolide is also considered the precursor of many sesquiterpene lactones with significant biological activities [17]. Expressed sequence tag (EST) sets from *Nicotiana benthamiana* led to the isolation of germacrene A synthase (*GAS*), germacrene A oxidase (*GAO*) and the recently characterized chicory costunolide synthase (*CiCos, CYP71BL3*) genes. These were co-expressed in *S. cerevisiae* WAT 11 strain to produce low yields of costunolide [17]. Costic acid is a related sesquiterpene carboxylic acid that shows an interesting cytotoxic activity against several phytopathogenic fungi [18]. The functional characterization and expression of germacrene A synthase/ germacrene A oxidase (*TcGAS/TcGAO*, *CYP71AV2*) and costunolide synthase (*TcCOS*) genes from *Tanacetum cinerariifolium* in yeast (*WAT11*), resulted in the production of α (6) and γ (7) costic acid isomers [19].

Zerumbone (8) is a humulene derivative with anti-inflammatory, anti-HIV and potent anti-tumoral properties that is abundant in *Zingiber zerumbet*. Zerumbone biosynthesis includes the generation of the 8-hydroxy-α-humulene intermediate. Recent studies revealed that *CYP71BA1* is the key enzyme involved in the biosynthesis of this molecule in accordance with its heterologous expression in yeast [20]. Co-expression of four genes from the mevalonate pathway including *CYP71BA1* and *ZSS1* in *Escherichia coli*, were able to produce small yields of 8-hydroxy-α-humulene [20].

These novel methods promise a major control on the biochemical synthesis of natural substances avoiding complex protocols for its induction and direct extraction from plant tissues. Table 1 describes the use of selected P450 for producing six sesquiterpenes with relevant biological activity.

Diterpenes

Some tricyclic and tetracyclic diterpenes produced by P450 enzyme activity are shown in Figure 2. Kaurenoic acid (9) is one of the most studied diterpenes with a wide range of biological activities [21]. Beyond the known participation of *ent*-kaurene oxidases (KO´s)

Table 1: Bioactive sesquiterpenes produced in heterologous systems using P450 from plant sources

P450*	Source	Bioactive product	Reference
CYP71AV1^{Y}	*Artemisia annua*	Artemisinin	[8,9]
CYP71D55^{Y}	*Hyoscyamus muticus*	Solavetivone	[15]
CYP71D20^{Y}	*Nicotiana tabacum*	Capsidiol	[14]
CYP71BL3^{Y}	*Nicotiana benthamiana*	Costunolide	[17]
CYP71BA1^{YB}	*Zingiber zerumbet*	Zerumbone	[20]
CYP71AV2^{Y}	*Tanacetum cinerariifolium*	Costic Acid isomers	[19]

**P450 genes involved in the biosynthesis of the bioactive product but not necessarily in the final step. Superscripts correspond to their expression in yeast (Y) or bacterial (B) systems, respectively*

from plants in gibberellin biosynthesis, current approaches highlight the potential uses of this family of P450 enzymes in several biotechnological areas. The records of the National Center for Biotechnology Information (NCBI) reveal at least 30 putative KO´s nucleotide sequences. However, the Braunschweig Enzyme Database (BRENDA) reveals that only about 17 % of those sequences have been biochemically characterized so far.

The non-sugar sweeteners global market was calculated to rise from $ 9.2 billion in 2010 up to US$ 9.9 billion in 2016 [22]. Considering the commercial importance of this market, the source for new procedures to get these products was also elicited. Stevia is a sweetener and sugar substitute extracted from the leaves of *Stevia rebaudiana*. The reconstruction of the steviol metabolic pathway in yeast, including the expression of a fungal KO and the *ent*-kaurenoic acid 13-hydroxylase (KA-13-H) genes is currently protected by a patent due to the commercial interest in the controlled production of natural glucosides [23]. Genetic engineering for steviol glycosides in yeast is considered a reliable option that may contribute to the optimization of this non-caloric sweetener for industrial aims. This new technology will change the perspectives on stevioside production because of their ecological advantages.

Novel KO-cDNA sequences from medicinal or agronomic plants, such as *Montanoa tomentosa* (*MtKO*), *Momordica charantia* (*McEKO*), *Cucumis sativus* (*CKO*) and the moss *Physcomitrella patens* (*CYP701B1*); were recently reported [24-27]. However, only *CYP701B1* and *MtKO* (*CYP701A-type*) were expressed in yeast and characterized at biochemical level, the respective enzymes showed resistance to relative high concentrations of azolic compounds [26,28]. This characteristic could further be used in metabolic engineering procedures. The apparent kinetic parameters of *MtKO* enzyme are also interesting considering that the high value of Km app supports aputative multisubstrate activity [28].

Genetic engineering of *ent*-kaurene and *ent*-kaurenoic acid in yeast was improved by using known KO enzymes from *Gibberella fujikuroi* and *Arabidopsis thaliana* [29]. The engineered yeasts containing these recombinant enzymes yield at least 0.5 grams L^{-1} of *ent*-kaurenoic acid in fed batch fermentation conditions. Deletions, peptide additions and codon optimization at the 5' end (N-terminal end) of the *AtKO1* gene from *A. thaliana* confer favorable changes in its specific activity [30]. These modifications have contributed to the expression of this P450 in relatively low-cost sustainable microorganisms such as *Escherichia coli*. In addition, these studies also show that KO possesses multifunctional activity on *ent*-beyerene and isokaurene skeletons. Further studies on the biochemistry of KO´s from medicinal plants that actively biosynthesize tetracyclic diterpenoids, could reveal interesting enzymatic properties for biotechnology use.

Abietadiene diterpenes present mainly in resins from conifers commonly show insecticide, allelopathic and antifungal effects [31]. Expression of the multifunctional and multisubstrate *CYP720B1* gene from *Pinus taeda* in yeast, demonstrates its capability for metabolizing abietadiene (10) and levopimaradiene (11) skeletons to originate oxygenated diterpenes associated with chemical defense [32]. The expression of *CYP720B4* gene from *Picea sitchensis* in the same system, also demonstrated its effectiveness in the oxidation of C-18 in several abietadienes, levopimaridienes and pimaranes (12),

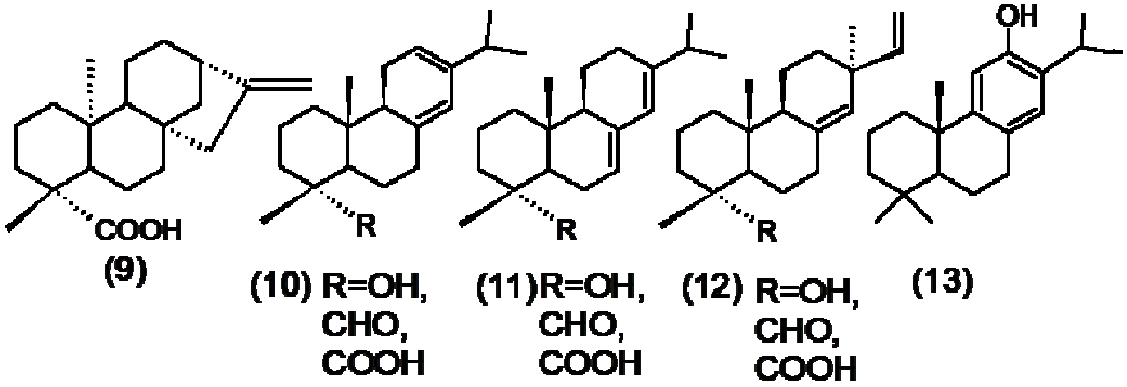

Figure 2: Some diterpenes derived from P450 enzyme activity

Table 2: Bioactive diterpenes produced in heterologous systems by using P450 from plant sources

P450*	Source	Bioactive product	Reference
CYP720B1^{Y}	*Pinus taeda*	Oxygenated abietadienes and levopimaradiene type compounds	[32]
CYP701B1^{Y}	*Physcomitrella patens*	*ent*-kaurenoic acid	[26]
CYP720B4^{Y}	*Picea sitchensis*	Oxygenated abietadiene and levopimaradiene type compounds	[31]
KA-13-H, CYP72-type ^{Y}	*Stevia rebaudiana*	Steviosides and rebaudiosides	[23]
CYP76AH1^{Y}	*Salvia miltiorrhiza*	Ferruginol	[33]
CYP701A-type^{Y}	*Montanoa tomentosa*	*ent*-kaurenoic acid	[28]

**P450 involved in the biosynthesis of the bioactive product but not necessarily in the final step. Superscripts correspond to their expression in yeast (Y)*

turning alcoholic into carboxylic acid forms [31]. The unusual multisubstrate activity of this enzyme reveals potential uses in pharmacology.

Ferruginol (13), an abietane diterpene (meroterpene type) with gastro-protective and anti-tumoral activities, has been produced at a yield of 10.5 mg L^{-1} in transformed *WAT11* strain (*S. cerevisiae*) containing *CYP76AH1* from *Salvia miltiorrhiza* and phyto-CYP reductase genes [33]. The discovery of this enzyme and its participation in tanshinone biosynthesis could be a relevant contribution to the elucidation of the biosynthetic pathway of carnosic acid, a pharmacologically active metabolite and also a chemotaxonomic marker of the *Salvia* genus. Yeast expression of P450 involved in abietane and piamarane type diterpenes represents the first approach for the generation of novel platforms for the diterpene resin acids controlled production. Table 2 shows recent P450 enzymes used for producing diterpenes with biological activity.

Triterpenes

Production of triterpenes with potential use in pharmacology has been improved in the last years (Figure 3). Initial attempts to clone P450 sequences involved in triterpene biosynthesis were carried out in Glycine max, with the identification of *CYP93E1* and heterologous gene expression in yeast. According to these studies β-amyrin and sophoradiol were transformed into olean-12-ene-3β-24-diol (14) and soyasapogenol B (15). Co-expression of *CYP93E1* and β-amyrin synthase genes from this plant in the *S. cerevisiae* system showed small yields of olean-12-ene-3β-24-diol [34].

Glycyrrhizin (16) is a triterpene glycoside sweetener biosynthesized by *Glycyrrhiza* spp. (licorice plants). Expression in yeast of *CYP88D6* gene from an EST bank of these plants showed its participation in the modification of β-amyrin into 11-oxo-β-amyrin, a putative intermediate in glycyrrhizin biosynthesis [35]. Subsequent studies revealed that *CYP72A154* enzyme, was able to oxidize 11-oxo-β-amyrin into glycyrrhetinic acid, which is a glycyrrhizin aglycone [36].

Medicago truncatula is a Mediterranean medicinal plant that actively produces hemolytic saponins. First biochemical studies of *CYP716A12* gene from this plant revealed its capacity for transforming β-amyrin and erythrodiol into oleanolic acid (17), a bioactive triterpene with hepatoprotective, antitumor and antiviral properties [37]. Interestingly, simultaneous studies confirmed that *CYP716A12* protein is a multifunctional enzyme able to produce ursolic acid (18) and betulinic acid (19) after expressed in yeast (INVSc1). Homologs of *CYP716A12* such as *CYP716A15* and *CYP716A17* from *Vitis vinifera* exhibited similar activities like *CYP716A12* [38].

Research on ginsenoside biosynthesis (*Panax ginseng*) reveal specific P450 enzymes involved in the addition of the aglycone portion of such molecules. The oxidation of dammarenediol-II at the C-12 position to protopanaxadiol (20) by *CYP716A47* in the yeast strain *WAT21* was reported [39]. Subsequent findings revealed that *CYP716A53v2* had the protopanaxadiol 6-hydroxylase enzyme activity. This one produces protopanaxatriol (21) from the final aglycone protopanaxadiol, which is posteriorly glycosylated to several ginsenosides [40]. Recently, *CYP716A52v2* was functionally characterized as a β-amyrin 28-oxydase involved in the biosynthesis of oleanane type ginsenosides [41].

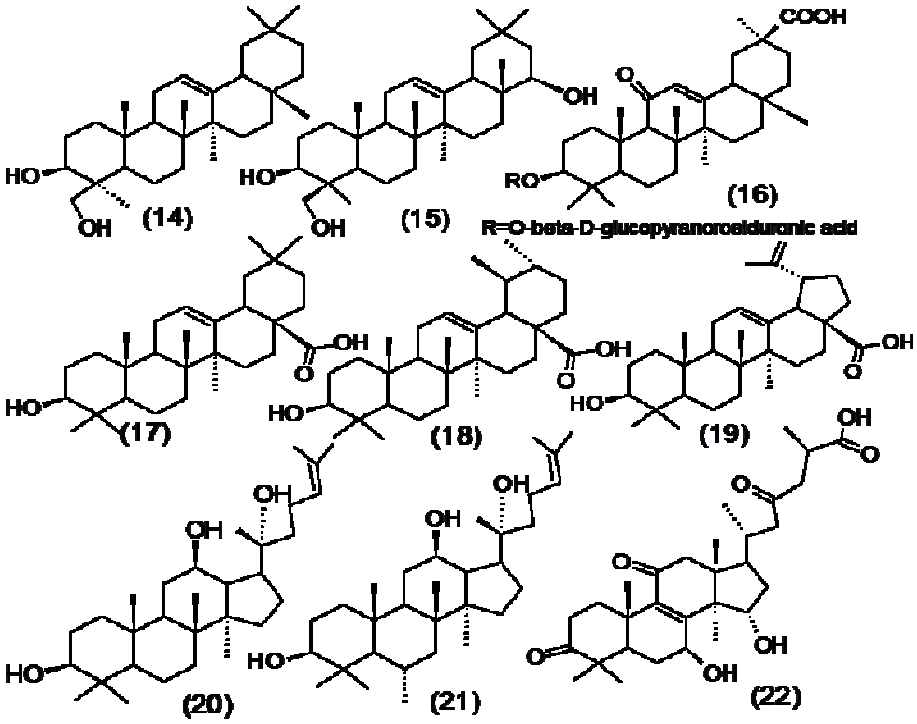

Figure 3: Some triterpenes derived from P450 enzyme activity

Table 3: Bioactive triterpenes produced in heterologous systems using P450 from plant sources

P450*	Source	Bioactive product	Reference
CYP93E1^{Y}	*Glycine max*	Olean-12-ene-3β-24-diol Soyasapogenol B,	[34]
CYP88D6^{Y}	*Glycyrrhiza spp.*	11-oxo-β-amyrin	[35]
CYP72A154^{Y}	*Glycyrrhiza spp.*	Glycyrrhetinic acid	[36]
CYP716A12^{Y}	*Medicago truncatula*	Oleanolic acid, Ursolic acid Betulinic acid	[37, 38]
CYP716A15^{Y}	*Vitis vinifera*	Ursolic acid Betulinic acid	[38]
CYP716A17^{Y}	*Vitis vinifera*	Ursolic acid	[38]
CYP716A47^{Y}	*Panax ginseng*	Protopanaxadiol	[39]
CYP716A53v2^{Y}	*Panax ginseng*	Protopanaxatriol	[40]
CYP716A52v2^{Y}	*Panax ginseng*	Oleanane type ginsenosides	[41]
GLCYP450^{Y}	*Ganoderma lucidum*	Ganoderic acids	[43]

**P450 involved in the biosynthesis of the bioactive product but not necessarily in the final step. Superscripts correspond to their expression in yeast (Y)*

Ganoderma lucidum, the millenary oriental fungus with medicinal and nutritional properties, biosynthesize ganoderic acid A (22) that shows a potent antioxidant activity [42]. Recent studies reported a GLCYP450 as a P450 probably involved in the biosynthesis of ganoderic acids [43]. Table 3 shows recent P450 enzymes used for producing triterpenes with biological activity.

GENETIC ENGINERING OF P450 INVOLVED IN FLAVONOID BIOSYNTHESIS

Some flavonoids with nutraceutical or pharmacological properties were produced in heterologous systems by using P450 coding sequences (Figure 4). Hydroxylation process is critical for flavonoids with dynamic activity. Significant efforts to produce flavanones in *S. cerevisiae* have been carried out [44]. Starting from natural precursors such as p-coumaric acid, cinnamic acid and caffeic acid, a yield of 28.3 mg L^{-1} naringerin (23), 16.3 mg L^{-1} pinocembrin (24) and 6.5 mg L^{-1} eriodictyol (25) respectively were obtained. In these findings naringerin and pinocembrin were produced 62 and 22 times more efficiently respect to previous attempts in prokaryotic cells.

The reconstruction of anthocyanin pathway inside *E. coli* has also been achieved [45]. The gene cluster used for this aim included the flavonoid 3' hydroxylase (F3H-P450)

anthocyanidin synthase (ANS) from Malus domestica and the flavonoid 3-O-glucosyltransferase from *Petunia hybrida*. Similar attempts to yield phenylpropanoids by using exogenous precursors and basic cDNA sequences for enzymes involved in flavonoid biosynthesis were posteriorly carried out in both *E. coli* and *S. cerevisiae* [46,47]. In these works the participation of flavonoid-3-hydroxylase (F3'H) and flavonoid-3-5-dihydroxylase (F3'5'H) displayed an essential role in the generation of the novel strains specialized in the production of flavonoids. Today F3'H and F3'5'H are key enzymes used in bringing about pigmentation changes in flowers and fruits in order to enhance their nutraceutical properties [48].

Leonard *et al* reported an interesting approach in engineered *E. coli* for the production of kaempferol (26) and quercetin (27), which are potent antioxidants and anti-obesity phenylpropanoids [49,50]. This attempt was carried out by reconstructing the basic flavonoid pathway which included the hydroxylating activity of flavonoid 3-β-hydroxylase from *Malus domestica* (*MdFTH*) and F3'5'H from *Catharanthus roseus*. The authors subsequently reported a significant improvement in flavonoid production by the insertion and overexpression of the very active acetyl-CoA carboxylase (PlACC) from *Photoharbdus luminicens* [51].

Vannelli *et al* described the production of p-hydroxycinnamic acid (28) from glucose in yeast [52]. The novel strain was achieved by using both phenylalanine and tyrosine ammonia lyases (*PAL, TAL*) genes from the fungus *Rhodotorula glutinis*. Both were simultaneously expressed with cinnamate-4-hydroxylase (*C4H-P450 monooxygenase*) and *P450 CYP* reductase genes from *Helianthus tuberosus*. Improvements in the production of p-hydroxycinnamic acid (up to 700 mg L-1) were reached in *Streptomyces lividans* using TAL and an endoglucanase from *Rhodobacter sphaeroides* [53]. Despite the latter advances, current engineered microorganisms for producing hydroxylated flavonoids require exogenous precursors to generate them. Therefore, a great challenge for flavonoid genetic engineering is to get self-sufficient microorganisms able to produce those metabolites without the addition of external intermediates. This condition has only been successfully achieved for relatively simple stilbene flavonoids such as resveratrol (29) in transgenic *E. coli* strains [54]. Table 4 shows some conserved P450 monooxygenases used for scale the production of hydroxylated flavonoids.

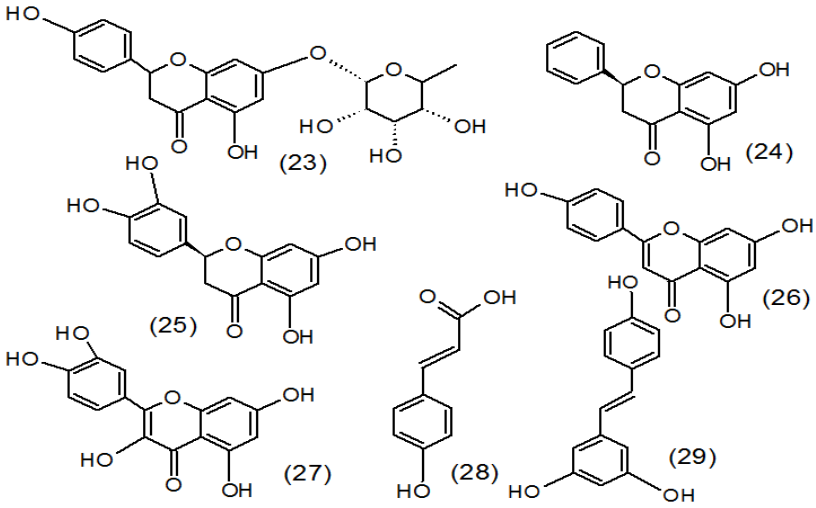

Figure 4: Some flavonoids derived from P450 enzyme activity

Table 4: Bioactive flavonoids produced in heterologous systems using P450 from plant sources

P450*	Source	Bioactive product	Reference
F3'H-CYP75B-type^{YB}	*Malus domestica*	Flavonones	[44-47]
F3'5'H-CYP75A8^{B}	*Catharanthus roseus*	Kaempferol, Quercetin	[49]
C4H-CYP73A1^{Y}	*Helianthus tuberosus*	p-hydroxycinnamic acid	[52]

**P450 involved in the biosynthesis of the bioactive product but not necessarily in the final step. Superscripts correspond to their expression in yeast (Y) and bacteria (B)*

GENETIC ENGINEERING OF P450 ENZYMES INVOLVED IN ALKALOID BIOSYNTHESIS

Advances in the synthesis of pharmacologically active alkaloids by genetic engineering have been reported (Figure 5). *Hyoscyamus niger* commonly known as blackhenbane, is an European and North American medicinal plant that biosynthesizes high concentrations of tropane alkaloids such as hyoscyamine (30). Li *et al* demonstrated the participation of *CYP80F1* in the rearrangement of (R)-littorine to (S)-hyoscyamine aldehyde, an intermediary in the biosynthesis of hyoscyamine [55]. Novel insights describe that *CYP80F1* catalyzes the isomerization and hydroxylation of littorine at the 3′-position [56]. *CYP719B1 gene* was isolated from *Papaver somniferum* and showed a salutaridine synthase identity. This enzyme catalyzes the conversion of (R)-reticuline to salutaridine (31), a crucial step in morphine biosynthesis [57]. Due to the medical importance of morphine (32), a specific transgenic opium poppy line was obtained by the insertion of *CYP80B3* ((S)-N-methylcoclaurine 3'-hydroxylase) gene, their over-expression resulted in > 400 % increase of the total alkaloid content in the plant [58].

CYP80G2 enzyme from *Coptis japonica* (*hilo de oro japonés*) catalyzes the C-C phenol for producing (S)-corytuberine (33) from (S) reticuline, The latter compound is a key precursor involved in morphinans, aporphines, pavines, protoberberines, protopines and benzophenanthiridines [59]. The golden poppy (*Eschscholzia californica*) is a Papaveraceae that actively grows in California (USA) and Baja California (México). The metabolism of this plant is meanly channeled to the biosynthesis of isoquinoline alkaloids. The yeast expression of *CYP719A5* gene showed its role as cheilanthifoline (34) synthase whereas *CYP719A9* enzyme catalyzed the methylenedioxy bridge of (R,S)- reticuline (35) [60].

Catharanthus roseus is considered the sole source of terpene-indole alkaloids for anti-tumoral therapy. According to novel evidence, the multifunctional *CYP71BJ1* enzyme is directly involved in the steroselective C19 hydroxylation of tabersonine (36), lochnericine (37) and vincadiformine (38) [61]. Table 5 shows recent P450 used to produce pharmacologically active alkaloids.

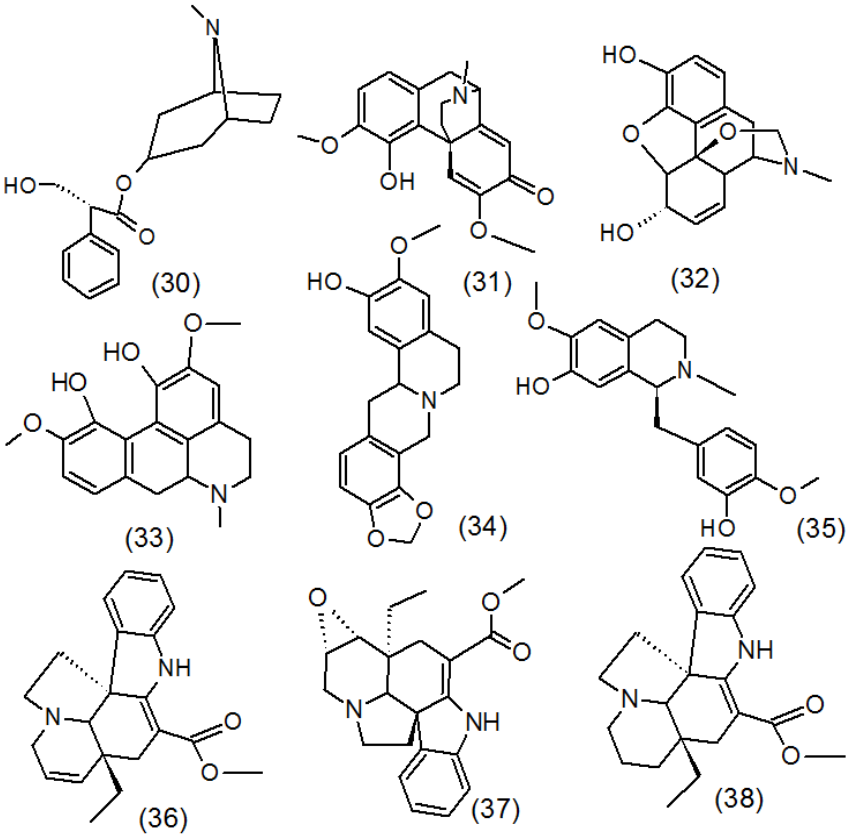

Figure 4: Some alkaloids derived from P450 enzyme activity

Table 5: Bioactive alkaloids produced in heterologous systems using P450 from plant sources

P450*	Source	Bioactive product	Reference
CYP80F1^{Y}	*Hyoscyamus niger*	Hyoscyamine	[55]
CYP80B3^{P}	*Papaver somniferum*	Morphine derivatives	[58]
CYP80G2^{Y}	*Coptis japonica*	(S)-corytuberine	[59]
CYP719A5^{Y}	*Eschscholzia californica*	Cheilanthifoline	[60]
CYP719A9^{Y}	*Eschscholzia californica*	(R,S)- reticuline	[60]
CYP719B1^{Y}	*Papaver somniferum*	Salutaridine	[57]
CYP71BJ1^{Y}	*Catharanthus roseus*	Tabersonine, Lochnericine Vincadiformine	[61]

**P450 involved in the biosynthesis of the bioactive product but not necessarily in the final step. Superscripts correspond to their expression in yeast (Y), bacteria (B) and plants (P)*

CONCLUSION

Biotechnological platforms have shown a significant advance in the past few years, especially in the synthesis of terpenes in heterologous systems. This undoubtedly demonstrates the potential uses of P450 enzymes as key steps for creating novel microorganisms that actively produce high amounts of plant natural products in a short time. This condition should help to reduce the cost of pharmaceuticals for treating public health problems. Up to date, the artemisinin production in engineered yeasts is the most concrete example of success in the heterologous production of terpenes [9]. Yeast is the preferred model for the induction of microsomal P450. However, genetic engineering of the amino-terminus have currently revealed promising results for expressing this group of enzymes in more reliable microorganisms as *E. coli* [30]. The number of P450 coding enzymes for triterpene biosynthesis has particularly increased in the last nine years. EST technology has significantly contributed to the availability of unique sequences from medicinal plant resources supporting the discovery of novel P450 with pharmacological potential.

Substantial advances have been achieved in the production of relatively simple flavonoids as the case of resveratrol (3,5,4'-trihydroxy-trans-stilbene) in *E. coli* [54]. Conversely, in many cases the microbial synthesis of hydroxylated flavonoids with a complex structure still depends on exogenous precursors to get enough yields.

Production of alkaloids and pseudo alkaloids in heterologous systems is another big challenge for synthetic biology, considering the exceptional reactions that they carry out and their diversity in the plant kingdom [3]. It is probable that functional genomics for alkaloid production is in full swing due to the recent studies validating their high scale production in genetically modified microorganisms as *E. coli* and *S. cerevisiae* [62]. Role of cytochrome P450 enzymes in alkaloids biosynthesis are quite specific compared with those involved in the biosynthesis of flavonoids and terpenes. Nonetheless, there are fascinating advances in the generation of transgenic lines of opium poppy for producing benzylisoquinoline derivatives with appreciated activity in the pharmaceutical market.

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