

Chemistry and evolution of the Piperaceae*

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Abstract: The chemistry of members of the family Piperaceae is of great interest owing to the variety of biological properties displayed. A survey of structural diversity and bioactivity reveals that groups of species specialize in the production of amides, phenylpropanoids, lignans and neolignans, benzoic acids and chromenes, alkaloids, polyketides, and a plethora of compounds of mixed biosynthetic origin. Bioassays against *Cladosporium cladosporioides* and *C. sphaerospermum* have resulted in the characterization of various amides, prenylated phenolic compounds, and polyketides as potential classes of antifungal agents. Studies on the developmental process in seedlings of *Piper solmsianum* have shown that phenylpropanoid are produced instead of the tetrahydrofuran lignans found in adult plants. In suspension cultures of *P. cernuum* and *P. crassinervium*, phenylethylamines and alkamides predominate, whereas in the adult plants prenylpropanoids and prenylated benzoic acids are the respective major compound classes. Knowledge of the chemistry, bioactivity, and ecology of Piperaceae species provides preliminary clues for an overall interpretation of the possible role and occurrence of major classes of compounds.

Keywords: Piperaceae; secondary metabolites; amides; chromenes; polyketides; lignans; mimetism.

INTRODUCTION

Biodiversity in the Neotropics is associated with a variety of biomes including the cerrado, caatinga, dry highlands, flooded areas, and Atlantic forest. Within these tropical and subtropical regions, some 2000 species representing the four major genera of the family Piperaceae, namely, *Piper*, *Peperomia*, *Sarchorhachis*, and *Ottonia*, are to be found [1–5]. Species of *Piper* are located in all types of vegetation but mostly as components of pioneer vegetation, while *Peperomia* species are quite commonly encountered as epiphytes or as succulent plants in moist forest and highland areas, respectively. Although general information relating to the biology of members of these genera is somewhat limited, some studies concerning the mechanism of pollination and seed dispersal have been carried out [6]. Indeed, the high reproductive trait of *Piper aduncum* is thought to be responsible for the disturbingly successful invasion of the lowlands of Papua New Guinea by this species [7].

The family Piperaceae, together with other members of the order Piperales such as the Aristolochiaceae, Saururaceae, and Lactoridaceae, has been classified among the basal Angiosperms. A detailed knowledge of characters relating to the secondary chemistry, biosynthesis, and biology could

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contribute to establishing the evolution of this challenging group. The taxonomy of the Piperaceae is also somewhat complex. Very recently, the phylogeny of *Piper* species has been addressed through studies of floral morphology in association with DNA sequence data derived from *rbcL*, *atpB*, and 18S [5], whilst that of *Peperomia* has been inferred from investigations of *trnK* and *matK* regions [8]. Such approaches have provided a clear relationship between species and clades, but it is not certain whether a complementary correlation exists between secondary compounds. Thus the identification of molecular traits should provide interesting possibilities for taxonomic projects.

The most significant compound of *Piper* species is the piperamide piperine, which constitutes the active pungent principle of black pepper (*Piper nigrum*) [4]. A large number of reports have demonstrated the remarkable potential of such amides as insecticides, molluscicides, and antifungal agents [9]. Together with amides, various other classes of phytochemicals have been isolated from *Piper* species, principally the chromenes [10,11], phenylpropanoids, lignans and neolignans [12–14], alkaloids [15], and several compounds of mixed-biosynthetic origin [4].

In comparison with the genus *Piper*, our knowledge of the chemistry of *Peperomia* is rather limited. Some structurally related compounds from *Peperomia*, including prenylated phenolics [16] and polyketide-chromenes [17,18], have been reported. Additionally, compounds derived from the shikimate pathway are also well represented in *Peperomia* species and these include the secolignans, the peperomins, and the styrene dimers derived from phenylpropanoid in which the γ -carbon is absent [19,20].

The aim of the present paper is to highlight the chemistry and bioactivity of selected secondary compounds isolated from members of the Piperaceae, and to consider their biosynthetic relationships in the context of the taxonomy of the family.

BIOACTIVE COMPOUNDS AND THEIR BIOSYNTHETIC ORIGINS

As part of a bioprospecting program relating to the isolation of bioactive compounds from Brazilian biodiversity, extracts from various species of Piperaceae have been screened using an antifungal bioassay. A number of extracts proved to be active against *Cladosporium sphaerospermum* and *C. cladosporioides* in a bio-autographic screen, and those derived from *Piper hispidum* [21], *P. arboreum*, and *P. tuberculatum* [22,23], were selected for dereplication studies. Chromatographic fractionation of these extracts yielded several classes of amides, amongst which the piperidone PT-4 and the piperidine PT-5 from *P. tuberculatum* were found to be highly active (Fig. 1). Additionally, the pyrrolidine-type amides PAR-1 and PAR-3 isolated from *P. arboreum* were extremely active with minimum inhibitory concentration (MIC) values of 0.1 μg in comparison with that of 0.5 μg exhibited by the positive control nystatin.

Together with the significant activity of the *Piper* amides against fungi, a large volume of evidence is available relating to their toxicity, synergistic effect, and specificity against insects [24,25]. Indeed, the amides represent one of the classes of natural products considered most promising as potential pesticides by virtue of their toxicity against a wide variety of microorganisms and insects [26–28]. In this context, the suggestion that *P. nigrum* could be used in the protection of crops and of stored products is noteworthy [24].

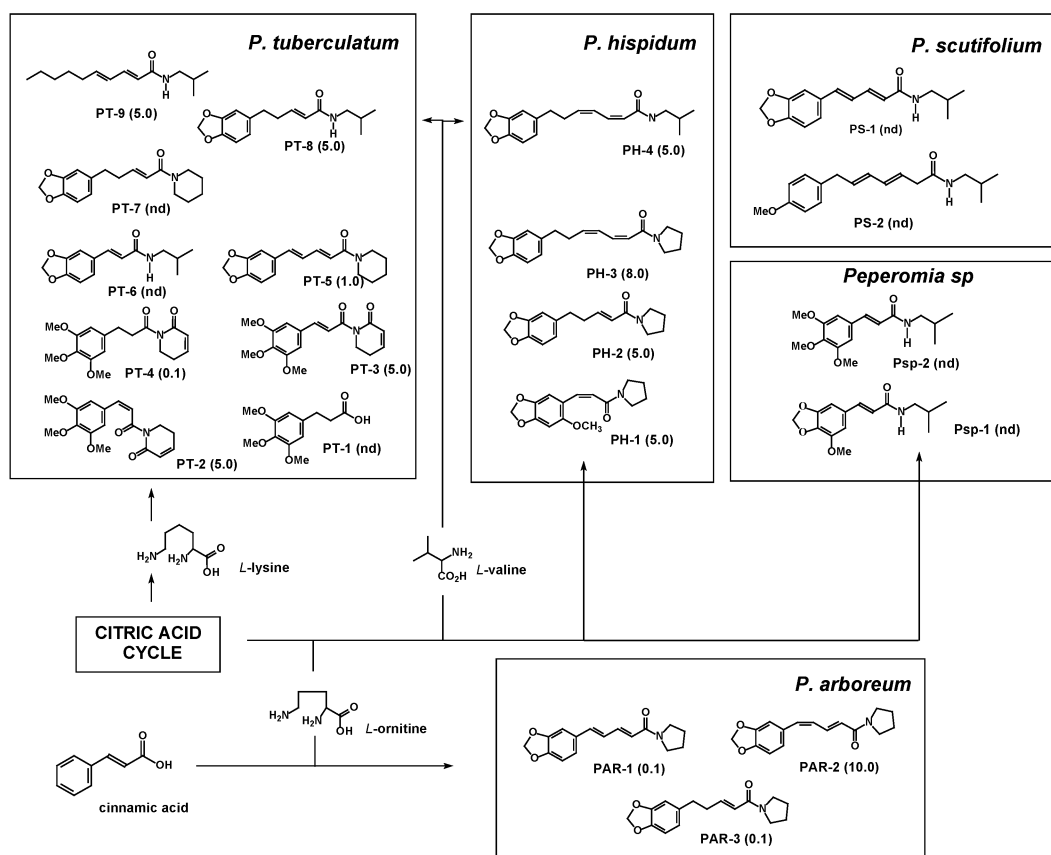


Fig. 1 Biosynthetic routes to amides isolated from *Piper* species and one species of *Peperomia*. The MIC (μg) values shown in parenthesis were determined against *C. sphaerospermum*: the positive control employed in the assay was nystatin, which presented a MIC value of 0.5 μg ; nd = not determined.

The occurrence of bioactive amides in *Piper* species is quite common [4], and we have detected several representatives in *P. hispidum* and *P. scutifolium* and also in one species of *Peperomia*. Additionally, chromenes, prenylated benzoic acid, hydroquinones, and polyketides with significant antifungal activities have also been isolated from *Piper aduncum*, *P. hostmanianum*, *P. crassinervium*, and *P. gaudichaudianum* (Fig. 2) [11]. A preliminary study of the structure–activity relationships indicated that the antifungal activity against *C. cladosporioides* is associated directly with the lipophilic properties of the molecules such as that occasioned by methylation of chromene I, or by the introduction of a second isoprenyl moiety as in chromene III. It is also worth mentioning that benzoic acid derivatives from several *Piper* species are active against microorganisms [29–31] and that, as with the amides, this class of compound appears to be characteristic of *Piper* species.

Some Piperaceae species also contain alkylated phenolic compounds formed by prenylation with dimethylallyl pyrophosphate (DMAPP), geranyl pyrophosphate (GPP), or farnesyl pyrophosphate (FPP). Such is the case for *P. aduncum*, *P. crassinervium*, and *Potomorphe umbellata*, in which prenylation involves either the introduction of a second prenyl group (chromenes I and II), or of geranyl (prenylated hydroquinone) or nerolidyl groups (4-nerolidylcatechol). For *Piper* species, the aromatic moiety in all of these cases has been shown to be *p*-hydroxybenzoic or protocatechuic acid [32] (Fig. 2). Interestingly, the hydroquinones isolated from leaves of *P. crassinervium* [11,33] bear prenyl moieties and represent natural products that have rarely been found in non-marine organisms [34,35]. The

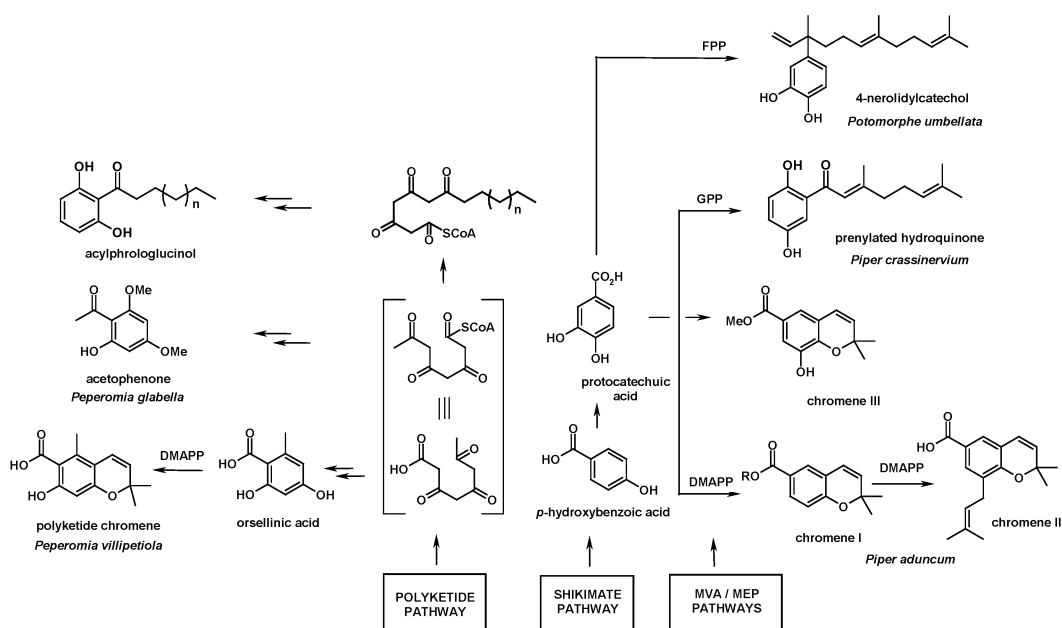


Fig. 2 Structural and biosynthetic relationships between the meroterpenes and polyketides present in members of the family Piperaceae. DMAPP = dimethylallyl pyrophosphate; GPP = geranyl pyrophosphate; NPP = nerolidol pyrophosphate; MEP = methylerythriol phosphate; MVA = mevalonic acid.

chelated phenolic function is, however, also present in the acylphloroglucinol isolated from *Peperomia glabella*, and this could be considered to be an example of chemical mimicry between the two species. The biological activities of these hydroquinones include inhibition of mitosis as well as antitumor, anti-leukemic [36,37], analgesic, relaxant [38], and antioxidant [39] effects. The significant antioxidant activity of prenylated hydroquinones, in comparison with hydroquinone itself, to inhibit lipoperoxidation is likely due to the presence of the lipophilic prenyl group that facilitates insertion of the molecule into the liposome in the model assay. Prenyated hydroquinones with a *2E*-configuration exhibited higher activities than their corresponding *2Z*-isomers, probably owing to better insertion into the liposome [40]. The presence of alkylated phenolic compounds in a number of *Piper* species suggests that this genus might represent a promising target in further searches for compounds with important antioxidant properties. Indeed, the remarkable antioxidant properties of 4-nerolidylcatechol are already being exploited in certain cosmetic preparations in an attempt to alleviate the symptoms of aging [41].

Our studies on antifungal compounds have also led to the isolation of several orsellinic acid-based chromenes such as that found in *Peperomia villipetiola*, which showed antifungal activity against *C. cladosporioides* [17]. Indeed, the structural similarity between the chromenes produced by species of *Piper* and *Peperomia* is impressive. However, the benzoyl moieties in the chromenes present in *Piper* species are produced from shikimic acid [42], whilst most of the aromatic moieties in the related compounds from *Peperomia* species, including the long alkyl chain phenols found in *P. villipetiola* and *P. serpens* [17,18], derive from the polyketide pathway. Virtually nothing is known concerning the possible selective pressures that could be responsible for the formation of such biomimetic compounds.

The discovery of bioactive compounds from *Peperomia* species based on popular medicinal uses of the plant are, unfortunately, very rare. Among the few examples that have been reported are the isolation of an antitumor dibenzylbutyrolactone derivative [43,44], and tetrahydrofuran lignans [45]. Several tetrahydrofuran lignans have also been isolated from *Piper solmsianum*, and these exhibit potent in vitro trypanocidal activities against trypanomastigote forms of *Trypanosoma cruzi*, the causative

agent of Chagas disease [46]. Since there is currently no efficient chemoprophylactic agent to prevent transmission of the disease by blood transfusion, the development of trypanocidal compounds is urgently required. Several tetrahydrofuran derivatives and analogs have been synthesized, and initial structure–activity relationships have been reported [47,48]. Among natural and tetrahydrofuran analogs, a *trans* relationship between the aromatic rings is necessary in order to maximize activity. Synthetic 1,4-bis-(3,4-dimethoxyphenyl)butane-1,4-dione presented an IC₅₀ value of 10 μM against Y strains of *T. cruzi*, a value that is comparable with that of the natural trypanocidal compound grandisin. Further investigations in this area are currently underway.

DYNAMICS OF SECONDARY METABOLISM DURING DEVELOPMENT AND DIFFERENTIATION

Since members of the Piperaceae represent such rich sources of bioactive compounds, a detailed knowledge of the dynamics of secondary compound biosynthesis during various stages of plant maturity would be of value. To this end, selected *Piper* species have been cultivated both in vitro (suspension cultures and differentiated plantlets) and in the field (seedlings and adult plants) in order to investigate changes in chemical composition during the developmental process (Fig. 3). In the case of *P. aduncum*, the major component, the phenylpropanoid dillapiol, was present together with apiol, myristicin, and elemicin in the volatile fractions derived from both seedlings and adult plants. Moreover, the major amides of *P. tuberculatum* were present in all tissues of the adult plants and in in vitro plantlets [49]. On the other hand, the two well-investigated species, *P. regnellii* and *P. solmsianum*, contained dihydrobenzofuran (conocarpan) and tetrahydrofuran neolignans (e.g., grandisin), respectively, as major compounds only in adult tissues [14,50]. The biosynthesis of (+)-conocarpan by an enzyme preparation obtained from the mature leaves of *P. regnellii* exhibited high enantioselectivity for the production of (+)-form [51]. A similar specificity was observed in *P. solmsianum* with respect to the conversion of 5-methoxyisoeugenol into (+)-4,4'-*O*-di-demethylgrandisin, which was then methylated to (+)-grandisin (unpublished results). In contrast to the above, seedlings of both *P. regnellii* and *P. solmsianum* contained dillapiol and apiol as major compounds similar to *P. aduncum*. The regulation of the dimerization process during plant development is not yet clear.

Among several *Piper* species for which cell suspension cultures were developed, those from *P. cernuum* and *P. crassinervium* produced detectable amounts of secondary compounds [52], the profiles of which indicated a clear biosynthetic divergence between suspension cells and field-grown plants. Thus, adult specimens of *P. cernuum* produced compounds arising from the phenylalanine ammonia-lyase (PAL) pathway, intermediates of which were elaborated to simple cinnamic acid derivatives and a series of lignans (Fig. 3). The major components produced by suspension cultures, however, derived from tyrosine decarboxylase (TYDC) and included tyramine and dopamine. In the case of *P. crassinervium*, the major products present in adult plants were formed by the prenylation of *p*-hydroxybenzoic acids to yield a number of hydroquinones (Fig. 2). Although the phenylethylamines tyramine and dopamine were not detectable in suspension cells of this species, four alkamides were isolated and characterized (PCRA-1/4). Since the alkamides require dopamine as precursor [53], it appears that the alternative TYDC pathway is predominant in suspension cells of both *P. cernuum* and *P. crassinervium*. Dopamine is not common as a natural product, but has been previously identified in *P. amalago* [4], and both phenylethylamines have been found in plants as cinnamoyl conjugates in *Piper* species [54,55] and as *N*-benzoyltyramine in *Aniba riparia* [56]. The physiological role of tyramine amides as phytoalexins has been recognized in tobacco, *Nicotiana tabacum* [57], and they have also been shown to be directly involved in suberization processes in wounded potatoes (*Solanum tuberosum*) [58]. The only indication of a regulatory role for such compounds in suspension cells of *P. crassinervium* is the finding that alkamide production is induced by UV light (unpublished results).

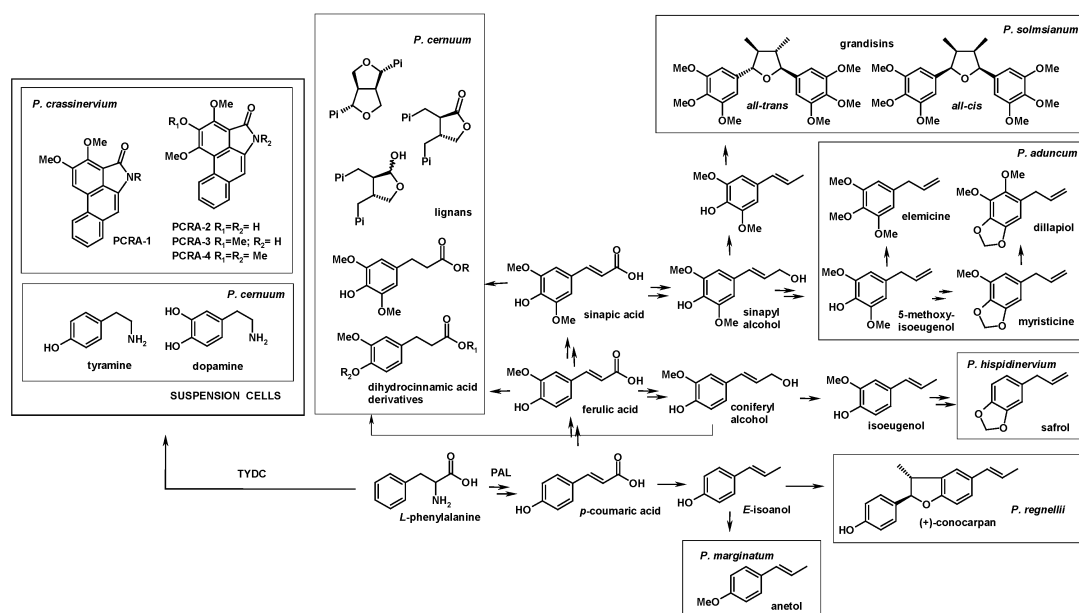


Fig. 3 Biosynthetic relationship between lignoids in *Piper* species and the divergence of secondary metabolism during differentiation of *P. cernuum* and *P. crassinervium*.

The prenylation of chromenes I to II in adult *P. aduncum* plants has been shown to change remarkably over each 24-h period, with the maximum level of prenyltransferase activity and the highest accumulation of both chromenes occurring regularly during the early morning (Fig. 4) [42]. Similar studies need to be conducted for the polyketide-chromenes in *Peperomia* species in order to understand how the prenylation step is regulated. The biosynthetic origin of the prenyl moiety has been investigated only in the case of 4-nerolidylcatechol from *Potomorphe umbellata* [32]. The labeled precursors [$3\text{-}^{14}\text{C}$]-*R,S*-mevalonolactone, [$\text{U-}^{14}\text{C}$]-glyceraldehyde-3-phosphate, and [$\text{U-}^{14}\text{C}$]-glucose were fed to leaves of the plant in order to determine conditions that would permit maximum uptake of tracer. Further experiments were conducted with [$\text{U-}^{13}\text{C}$]-glucose as precursor, and the positions of label incorporated into 4-nerolidylcatechol were determined by ^{13}C NMR spectra analysis. The pattern of specific incorporation into the product (Fig. 5) was consistent with the simultaneous operation of the mevalonate and triose/pyruvate pathways, and suggested an exchange of plastidic and cytoplasmic isopentenyl diphosphate (IPP) units, respectively. Detailed investigations are under way in order to determine a general pattern of terpenoid biosynthesis in *Piper* species.

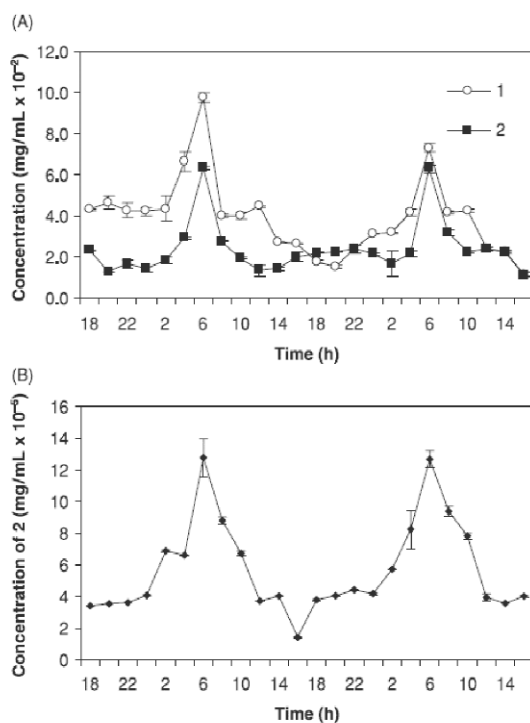


Fig. 4 Circadian changes in the content of chromenes **1** (○) and **2** (■) (A) and in the prenyltransferase activity in leaves of *P. aduncum* (B) [42].

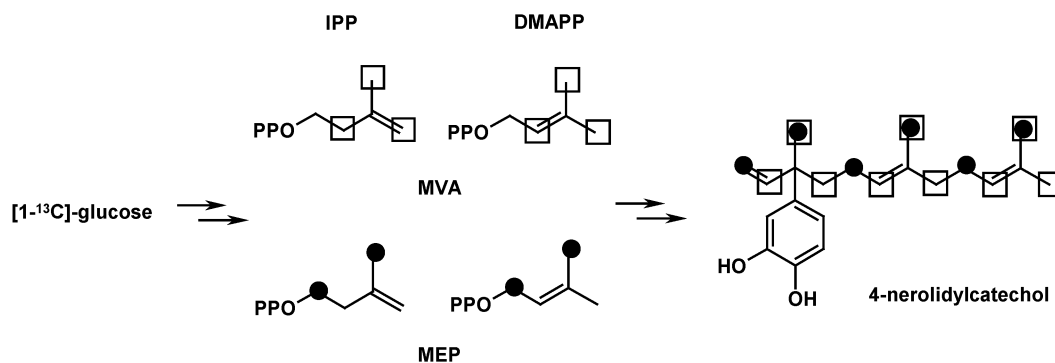


Fig. 5 Labeling pattern determined in 4-nerolidylcatechol biosynthesized from [1-¹³C]-glucose by *P. umbellata* indicating the participation of both mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways [32].

CONCLUSIONS AND FUTURE PROSPECTS

The chemical profiles of the species of *Piper* and *Peperomia* that have been investigated so far reveal biosynthetic lineages in the production of the major constituents. Compounds showing antifungal activities were isolated using bioactivity-guided procedures and subsequently characterized as amides, chromenes of different origin (shikimate and polyketide), and alkylphenols (polyketides and mero-terpenes). With respect to chromenes and alkylphenols, the structural similarities of the compounds found in various species of the two genera are suggestive of similar physiological roles, but the selective pressures that might be responsible for such convergence are not clear. Tetrahydrofuran lignans

have also been found in species of both *Piper* and *Peperomia*: those from *P. solmsianum* have been shown to be active against *T. cruzi* and their skeleta have been employed as scaffold structures for synthetic and structure–activity studies. The production of lignans in *Peperomia* is curious since seco-lignans (peperomins) and dinorlignans (pellucidins) have been detected in both *P. glabella* and *P. pellucida*: the structures of peperomins and dibenzylbutyrolactone lignans are also indicative of convergence.

A study of the dynamics of biosynthesis of the major secondary compounds in Piperaceae species revealed a circadian rhythm in the production of the chromenes in *P. aduncum* with the prenylation activity being controlled by light. Moreover, the production of tetrahydrofuran lignans and dihydrobenzofuran neolignans in *P. regnellii* and *P. solmsianum* appeared to be highly regulated during plant development since no production of lignans/neolignans was observed at the seedling stage. On the other hand, the seedlings produced highly oxygenated phenylpropanoids (dillapiol and apiol) as exemplified by *P. aduncum*. The analysis of differentiated and undifferentiated tissues of *P. cernuum* and *P. crassinervium* revealed a clear pattern of production of phenylethylamines and alkamides in cell suspension cultures, respectively.

In summary, knowledge relating to the plasticity of secondary metabolism and its regulation, together with information concerning the biological roles of the products, could provide a basis for the determination of taxonomic markers within the family Piperaceae.

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