

Contribution to the phytochemical study and biological activity of plants of Cuban flora

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ABSTRACT

Interest in natural products as a source for innovation in drug discovery and agrochemicals is still growing worldwide. Natural products, whose immense diversity has been appreciated for many years, may become in a rich source of novel chemical structures. Our country is a rich source of both biological and chemical diversity which may be useful as a source of novel chemical structures. Even when natural products have been used as medicinal agents for many years in Cuba, their use as agrochemicals are still limited. Thus, the present review focuses on recent advances in the studies on natural products performed by the "Centro de Estudios de Productos Naturales, CEPN" during the past ten years, highlighting on those with potential use as biomedical and agrochemicals. 15 plant species were studied; *Agave brittoniana* Trel. Subsp. *brachypus* A Álvarez de Zayas (Agavaceae); *Juniperus barbadensis* L. var. *Lucayana* (Britt.) RP Adams (Cupressaceae); *Melia azedarach* Linn. (Meliaceae); *Tectona grandis* Linn. f. (Lamiaceae); *Lantana camara* Linn. (Verbenaceae); *Lantana trifolia* Cham. (Verbenaceae); *Citrus sinensis* (Linn.) Osbeck cv Valencia (Rutaceae); *Maytenus buxifolia* (A Rich) Griseb (Celastraceae); *Maytenus elaeodendroides* Griseb (Celastraceae); *Maytenus urquiola* B Mory (Celastraceae); *Solanum americanum* W. Mill (Solanaceae); *Thalassia testudinum* Kon. (Hydrocharitaceae); *Sesbania rostrata* Bremek & Oberm (Fabaceae); *Pluchea carolinensis* G Don (Asteraceae) and *Ageratina havanensis* (HB & K) RM King & Robinson (Asteraceae). By means of a bio-guided fractionation, isolated structures as well as semi-purified fractions were assayed as antioxidant, antiparasitic, antiparasitic, antibiotic, insecticides, antifungal, and skin regenerating and healing effects. The strategy employed allows us to discover novel secondary metabolites with potential for industrial development as pharmaceuticals and/or agrochemicals. Besides, in addition to the goal of discovering new bioactive agents, the presence of unique bioactive compounds notably contribute to increase the knowledge of plant species in Cuba.

Keywords: bioactivity of Cuban plants, flavonoids, saponins and steroidal sapogenins, terpenoids and triterpenoids, quinones and phenolic compounds

Introduction

Natural products from plants continue as a source for innovation in drug discovery. Scientists are still exploring terrestrial and marine organisms for potentially valuable medical products since they have developed biochemical and physiological mechanisms that include the production of bioactive compounds for their protection. Many of these metabolites may play a general cellular role and thus they can be useful for their pharmacological action. In many cases, natural products have provided compounds as clinical/ marketed drugs, or as biochemical tools that demonstrate the role of specific pathways in disease and the potential of finding drugs.

Several investigations have described many molecules from natural sources that due to their relative low side effects and high efficacy, in comparison with those obtained from chemical synthesis, can be useful in the treatment of several human diseases [1, 2]. During 2001 and 2005 about 23 compounds useful to

improve the therapy in cancer, diabetes, and atypical dermatitis and neurodegenerative diseases have been obtained by pharmacology research in the discovery and development of novel natural compounds. Also, they have been studied to expand the treatment of bacterial, fungal and immunosuppressive therapy [3, 4]. However, natural product compounds not only serve as drugs or templates for drugs directly, but in many cases they can be useful as bioplaguicides [5, 6].

Our country is a rich source of both biological and chemical diversity. This diversity may be useful as a source of unique chemical compounds with the potential for industrial development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes and agrochemicals. Despite this, researches in the use of natural products as pharmaceutical agents are still limited. While our large biodiversity offers a wide resource for novel compounds, it also represents a great challenge for researches that requires inputs from va-

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rious scientific areas to bring this chemical diversity up to its therapeutic use. Accordingly, one of the major aims of the team of phytochemistry from the Centro de Estudios de Productos Naturales (CEPN), Faculty of Chemistry, University of Havana, has been to develop scientific projects focused on the identification of new compounds with potential for industrial development as pharmaceuticals and/or agrochemicals.

The purpose of this review is to highlight some of the principal findings obtained during the past ten years by our group, with special reference to the biomedical and agrochemical potential of new structures as well as semipurified fractions. With this aim, antioxidant, antiparasitic, insecticides, antifungal and skin regenerating effects were studied in 15 plant species that in some cases had not been previously explored.

Results and discussion

The most important results are shown in table 1 and figure 1. Table 1 shows the plants under study, the biological activity as well as the isolated metabolites from each plant species. The plants were selected according to chemotaxonomic, ethno-botanic and availability criteria. Figure 1 shows 25 novel metabolites isolated from some of these plants. Besides, metabolites produced by the fungi *Botrytis cinerea* Pers. and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., are also shown.

Isolation and characterization of flavonoids

Flavonoids are a family of metabolites widely distributed in nature. They are commonly found in plants, fruits, grains, among others. Flavonoids exhibit an extensive variety of beneficial biological activities in mammals, including its potent antioxidant capacity. We have isolated several flavonoids from different plant species such as: *Thalassia testudinum* Kon., *Pluchea carolinensis* (Jacq.) G Don, *Citrus sinensis* (Linn.) Osbeck c. v. Valencia, *Lantana camara* Linn., *Lantana trifolia* Cham, *Juniperus barbadensis* L. var. *Lucayana* (Britt.) RP Adams and *Ageratina havanensis* (H B & K) RM King & Robinson.

The ethanolic extract from *Thalassia testudinum* has been found to exhibit strong skin regenerating effect when applied topically. Bioassay-guided fractionation of the plant extract resulted in the isolation of thalassiolin B, a sulphated flavone glycoside that markedly reduces skin UVB-induced damage and exhibits radical scavenging activity on DPPH. This suggests that thalassiolin B is responsible for the skin regenerating effects of the crude extract of *T. testudinum* [7].

Antibacterial and antioxidant effects were observed in crude extract from leaves of *Pluchea carolinensis* (Jacq.) G Don, from which four flavonoids were isolated. When assayed, the isolated metabolites also exhibited antioxidant effects which suggest that these compounds are involved at least partially, in the pharmacological action of the extract [8, 9].

The seed extract from the species *Citrus sinensis* (Linn.) Osbeck c. v. Valencia was also investigated. A new flavone and another one previously described were isolated from the n-butanol extract. This extract was found to exhibit allelopathic activity against black glumelas red rice [10]. The species *Lantana camara*

Linn widely distributed in our country was also selected due to its previously reported allelopathic effects. In this study, the composition of its essential oil was determined as well as its insecticide effects [11]. In addition, a flavonoid was isolated from the leaves extract of this plant that showed a powerful antifungal activity against the fungus *C. cassicola*, similar to those found for the commercially available product LOGRAM [12]. Previous reports have shown allelopathic activity of the species *Lantana trifolia* Cham. Thus, in order to investigate such effect, several kinds of extracts from the leaves were studied. Two flavones were isolated and characterized from the most active extract [13]. The allelopathic effects of the species *Ageratina havanensis* (HB & K), was also under study, bearing in mind our previous results as well as the high content of flavonoids and glycosides. In this study, several extracts were used and two flavones and three flavones glycosides were identified, being two of them novel metabolites. In this study one of the isolated flavone showed allelopathic activity [14].

Saponins and steroidal saponins

Saponins are also widely distributed in nature, occurring primarily in the plant kingdom. Steroidal saponins consist in one or more monosaccharide moieties bonded to a non polar aglycone, which in this case is of steroidal nature. Steroidal saponins are present in different families of plants, such as *Solanaceae* and *Agavaceae*. These types of compounds have diverse range of biological properties.

Agavaceae sp are known to contain a high content of saponins and sapogenins. Thus, 12 saponins were identified and characterized from the plant *Agave brittoniana* Trel. subsp. *Brachypus*, an endemic species commonly found in the central region of Cuba. Their complex structures were elucidated by extensive spectroscopic techniques of NMR and MS, such as: 1D TOCSY and 1D ROESY, g-HSQC, g-HMBC, g-HQC-TOCSY and ESI-MS). The structural elucidation of these metabolites is considered an important contribution for the structural determination of saponins. In this study, a new method useful to identify the total content of saponins in crude extract and semipurified fractions was established and successfully used [15]. Several saponins were evaluated as antiparasitic agents against *Fasciola hepatica*, *Trichomonas vaginalis* and *Tripinosoma cruzi*. The results demonstrated that spirostane saponins showed the strongest activity when compared with the furostanics [16]. The best results were observed when they were assayed against miracidies.

The antibacterial and healing activity of the plant *Solanum americanum* W. Mill was also investigated bearing in mind its traditional used in folk medicine. Two glycoalkaloids, solanine and solamargine, and a new steroidal glycoside were isolated from this species. The healing activity was found for semipurified fractions as well as for solanine. Our results suggest the potential use of this glycoalkaloid in the treatment of uterus lesions [17].

An steroidal sapogenin was isolated from *Lantana trifolia* Cham for the first time, and it was also the first report from natural source [13]. This sapogenin was isolated from a fraction with strong allelopathic

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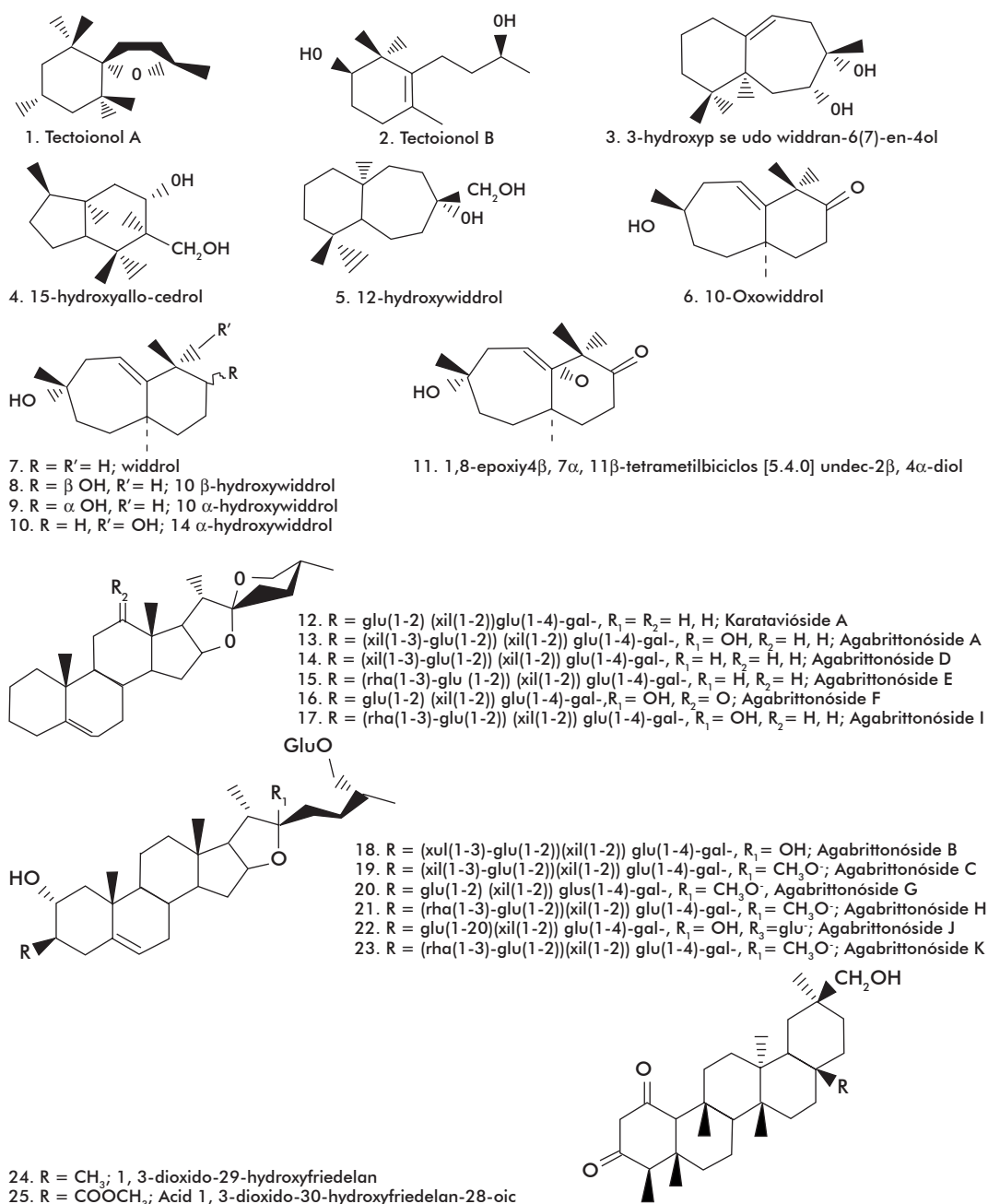


Figure 1. Structures of some novel metabolites isolated from Cuban plants.

effects and recently synthesized by the CPEN group, which demonstrated its capacity for inhibiting vegetable growth. Moreover, a novel protolimonoid, identified as 21 β -etoxymelianodiol and the 3-methoxy-4-hydroxybenzene acid were isolated from the fruit extract of *Melia azedarach* Linn, the second metabolite feeding deterrent activity when assayed against *Mocis latipe* and *Spodoptera fungiperda* [18, 19].

Terpenoids

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene, CH₂=C(CH₃)-CH=CH₂. Terpene hydrocarbons therefore have molecular for-

mula (C₅H₈)_n and they are classified according to the number of isoprene units. This metabolite group is well known to exhibit a broad range of biological properties.

A bioassay-guided fractionation of the wood and leaves extract of *Juniperus barbadensis* L. var. *Lucaiana* (Britt.) RP resulted in the isolation of two flavonoids and nine sesquiterpenes. Four of them showed antifungal activity against *Botrytis cinerea*, including three novel metabolites [20]. A compound with antifungal activity produced by the fungi *Botrytis cinerea* and *Colletotrichum gloeosporoides*, and four new metabolites were isolated and characterized when the mechanism of widdrol detoxification was studied. None

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Table 1. Studied plants, kind of proven activity and metabolites identified

Species studied	Kind of proven activity	Metabolites isolated and identified
<i>Juniperus barbadensis</i> L. var. <i>lucayana</i> (Britt.) RP Adams (Cupressaceae); (wood and foliage)	Antifungal for <i>Botrytis cinerea</i> Pers control	Wood: cedrol**, α -bisabolol**, widdrol**, other three sesquiterpenes, two flavones, 3-hydroxypseudowiddran-6(7)-en-4-ol**, 15-hydroxyallo-cedrol*, 12-hydroxywiddrol*, ** Foliage: sandaracopimaric acid ** and terpenes
<i>Melia azedarach</i> Linn.; (Meliaceae); (fruit)	Insecticide to larvae control of <i>Mocis latipes</i> and <i>Spodoptera fugiperda</i>	21 β -ethoxymelianodiol*, 3-methoxy-4-hydroxybenzoic acid**.
<i>Lantana trifolia</i> Cham.; (Verbenaceae); (foliage)	Allelopathic on etiolated coleoptile of wheat, lettuce, tomato and other	Two hydroxylates flavones, β -sitosterol, 25R-spyrostan β -3,5 α ,6 β -triol*,
<i>Citrus sinensis</i> (Linn.) cv Valencia (Rutaceae); (seeds)	Allelopathic against black glumelas red rice	6,7,8,3',4'-pentamethoxyflavone, 5,6,7,8,3',4'-hexamethoxyflavone*
<i>Lantana camara</i> Linn.; (Verbenaceae)	Insecticide, nematicide, antifungal	7-O- β -D-pectolinارينجين**, composition of essential oils, two flavonoid glycosides
<i>Maytenus buxifolia</i> (A Rich.) Griseb. (Celastraceae)	Allelopathic against black glumelas red rice, insecticide and antileishmanic	three nortriterpene quinone methides and two 1,3-friedelane diketones, one of them is novel
<i>Thalassia testudinum</i> Banks ex König, (Hydrocharitaceae)	Anti-inflammatory, antioxidant and tissue regenerative	Thalassiolin A, thalassiolin B** (regenerative effect of tissues), thalassiolin C, p-hydroxybenzoic acid
<i>Maytenus elaeodendroides</i> Griseb (Celastraceae) (stem bark)	Antifeedant on <i>Sitophylus orisa</i>	Six triterpenes of lupeol and friedelane group and methyl ester of 1,3-dioxo-30-hydroxyfriedelane-28-oic acid*
<i>Maytenus elaeodendroides</i> Griseb (Celastraceae) (stem bark)	<i>Maytenus elaeodendroides</i> Griseb (Celastraceae) (stem bark)	Six triterpenes of lupeol and friedelane group and methyl ester of 1,3-dioxo-30-hydroxyfriedelane-28-oic acid*
<i>Ageratina havanensis</i> (HB & K) RM King & Robinson; (Asteraceae); (leaves and stem)	Allelopathic with reduction of root and coleoptile growth on lettuce seedlings	Sakuranetine***, 7-methoxyaromadendrine**, **** and three glycosides, two of them are novel in the genus
<i>Solanum americanum</i> W Mill; (Solanaceae)	Healing and anti-inflammatory effect (cervicities of the cervix uteri)	Solasonine, solasodine, solamargine, tigogenine
<i>Pluchea carolinensis</i> G Don; (Asteraceae)	Antioxidant and antibacterial	Isorhamnetin, 3-O-isorhamnetin sulfate, eupalitin***, 3-methoxyquercetagine***
<i>Tectona grandis</i> Linn. f.; (Lamiaceae)	Phytotoxic and allelopathic on etiolated coleoptiles of wheat, lettuce, tomato and others	Forty metabolites from terpenes and quinones family, seven of them are novel
<i>Agave brittoniana</i> Trel. subsp. <i>brachypus</i> A Álvarez de Zayas; (Agavaceae)	Antiparasitic against <i>Trichomona vaginalis</i> , <i>Tripanosoma cruzi</i> , <i>Fasciola hepatica</i>	Six saponins, β -sitosterol, and twelve saponins: kataraviósido A, agabrittonosides A*, **, B*, C*, D*, **, E*, **, F*, G*, H*, I*, J*, K*
<i>Serbania rostrata</i> Bremek & Oberm (Fabaceae)	Antifeedant on <i>Sitophylus orisa</i> and Allelopathic (black glumelas red rice)	Mainly triterpenes and tannins

Metabolite described for the first time (new); **Metabolite that showed activity; ***Isolated for the first time in the genus. Of all metabolites isolated from these plants, thirty are described for the first time in literature (novel).

of them preserve the antifungal activity, which suggest that 12 and 4 positions of the molecule are essential for this activity, indicating the possibility to obtain bioactive analogues by synthetic transformations [21]. This hypothesis was further confirmed when nitrogen functions were bonded to these positions [22]. Furthermore, the crude n-hexane extract of the leaves of *Lantana trifolia* Cham showed allelopathic activity and a phytosterol and diterpene were isolated [23].

Several terpenoid compounds such as: one monoterpene, 7 bisnor-sesquiterpenes, one derivative by degradation of carotene, 4 sesquiterpenes and 8 diterpenes were isolated and characterized from the dry leaves of *Tectona grandis* Linn. Furthermore, seven apocarotenoids were obtained from a bioactive fraction from *Tectona grandis* Linn. f. two of them had not been previously reported as natural products (tectoionol A and B). Their complex structures were elucidated by inter-

pretation of 1D and 2D NMR, whereas the absolute configuration of sectional A was determined by means of the modified methodology of Mosher [24, 25].

Triterpenoids

Triterpenoids were found in the species *Maytenus buxifolia* (A. Rich.) Griseb. *Maytenus elaeodendroides* Griseb and *Tectona grandis* Linn.f. Three friedelane triterpenes were isolated from the back root of *M. buxifolia*, two of them with similar structure of meliten-quinones, which are chemotaxonomic markers in this genus. One of these structures had not been described before. Besides, two dimeric sesquiterpene-triterpenes were also isolated and they showed allelopathic activity [26].

Six metabolites from lupeol type, and three friedelane triterpenes as well as a diketone as novel triterpenes were isolated from the stem back extract of

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endemic species *Maytenus elaeodendroides* Griseb. This diketone showed feeding deterrent activity on *Sitophilus oriza*, a plague that causes significant damage to rice growing [27, 28].

On the other hand, seven triterpenes (four pentacyclic triterpenes of the lupane serie: lupeol, betulin, betulinic aldehyde and betulinic acid) [25] were isolated from the leaves extract of *Tectona grandis* Linn. F.

Quinones and phenolic compounds

The natural quinones are diketones, whose reduction product turns into a polyphenol. They are classified in benzoquinones, naftoquinones and quinone isoprenoids. In general, this compounds exhibit significant antioxidant capacities.

Two quinones and two phenolic compounds were isolated from *Tectona grandis* Linn. f., which included a novel quinone, named naftotectone A. This compound exerted the highest phytotoxicity of all isolated metabolites. The high pharmacological potential as well as the high concentration in the extract may account for the phytotoxic effect of this compound. Furthermore, seven apocarotenoids were obtained from a bioactive fraction of *Tectona grandis* Linn. f., two of them isolated for the first time from a natural source (Tectoionol A and B). On the other hand, Naftotectone A, 2-oxokovalenic acid, 19-hidroxyferruginol and the bisnor-sesquiterpene β 3, 9-dihidroxy-7,8-dihidro- β -ionol were isolated from this plant exhibiting the strongest phytotoxic activity [29].

The novel phenolic compounds (10-oxowiddrol, 10 α -hidroxywiddrol, 10 β -hidroxywiddrol y 14 α -hidroxywiddrol), were isolated from wood and leaf extracts of *Juniperus barbadensis* L. var. *Lucaiana* (Britt.) R. P. Adams, by bio-guided fractionation against the fungus *Botrytis cinerea* Pers., as well as studying the biotransformation of widdrol by the fungi *Botrytis cinerea* Pers. and *Colletotrichum gloeos-*

porioides (Penz.) Penz. & Sacc. Besides, in order to improve the antifungal activity, two other compounds were synthesized by chemical modification of 12-hidroxywiddrol.

Conclusions

A systematic work has been developed by the CEPN in the recent 10 past years regarding the phytochemical characterization of several Cuban plants, which are broadly distributed in Cuba. In this work, 15 species of plants were used. By means of bio-guided purification procedures, more than 30 novel compounds were isolated, purified and characterized. Flavonoids, steroidal saponins, saponinins, terpenes and quinones have been found as the major metabolites in these plants, which were identified by a combination of advanced spectroscopic methods. Among the identified compounds, several showed vigorous antioxidant, antiparasitic, antibacterial, skin regenerating as well as healing activities with potential use as pharmaceuticals. Also, antifungal, insecticide and allelopathic effects have been found in some of the new structures that may be useful for agrochemicals. In addition to the goal of discovering new bioactive agents, the project not only has contributed to collect data that will allow others to further study of the potential of several plant species living in Cuba, but also from the academic point of view, it has notably increased the scientific knowledge of the scientists and students engaged in the project. Currently, the project has given the opportunity to several graduated, eleven Masters in Science students and three doctoral students to conclude their Thesis.

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