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Natural products-based discovery of antitubercular agents from Philippine medicinal plants — A review

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Tuberculosis is one of the leading causes of death worldwide, more than HIV and malaria. New TB cases are emerging, including multidrug-resistant TB. The development of antimycobacterial assays led to the discovery of secondary metabolites that elicit promising inhibitory activity against *Mycobacterium tuberculosis*. This review covers literature published from 1999 to 2014 about natural products from Philippine medicinal plants with reported growth inhibitory activity in vitro against *M. tuberculosis* H_{37}Rv. The antitubercular compounds were grouped according to plant family and/or chemotype. Some exhibited structural significance but with low inhibitory activity (MICs of >128 μg/mL). While other previously reported compounds that were re-isolated exhibited anti-TB activity.

Keywords: antitubercular, anti-*Mycobacterium tuberculosis*, Philippine medicinal plants, natural products

Introduction

The World Health Organization listed tuberculosis as one of the top ten causes of death worldwide in 2015, and was responsible for more deaths than HIV and malaria [1]. It is a leading killer of people living with HIV, causing 35% of all deaths. Over 95% of TB deaths occur in low- and middle-income countries. In 2015, an estimated 480,000 people developed multidrug-resistant TB (MDR-TB). The TB incidence has dropped by an average of 1.5% per year since 2000, through TB diagnosis, the adoption of directly observed treatment short course (DOTS), and overall, the "End TB Strategy" recommended by WHO, to achieve the global TB target set by 2030 among the newly adopted Sustainable Development Goals. However, MDR-TB has been prevalent in all countries surveyed. In 2015, an estimated 480,000 people developed multidrug-resistant TB (MDR-TB). In the Philippines, an estimated 2.6% of TB cases are with MDR/RR-TB (rifampicin-resistance TB) **[**1**]**. Indeed, many are plagued with the disease, for a variety of reasons, foremost of which is poverty.

With the development of inhibitory-based anti-mycobacterial assays, a number of secondary

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metabolites from medicinal plants have been documented to elicit promising inhibitory activity against *Mycobacterium tuberculosis* [2–4]. Natural products and their derivatives have been shown to exhibit growth inhibitory activity against *M. tuberculosis* and some have been selected as inspiration molecules for the creation of new generation antituberculosis agents [4, 5]. Several review papers describing the discovery of antimycobacterial plant natural products have been published in the literature from 1990 to 2012 [6–8].

The Philippines is rich in medicinal plants, much of which remains unexplored, and thus, underutilized. Though more than 800 species of Philippine plants are considered medicinal, there is much more to be explored. Taking advantage of the diversity of our terrestrial plants, the Phytochemistry Group of the University of Santo Tomas Research Center for the Natural Sciences (UST-RCNAS) has committed itself to the exploration and utilization of these plants. The objective of the group has been to provide a scientific rationalization for the use of plants as sources of medicine against TB, either as phytopharmaceuticals or as new drugs for development.

The in-depth phytochemical investigation is conducted after a random or ethnobotanical selection of plants followed by screening of the crude plant extract against *M. tuberculosis* H_{37}Rv for bioactivity. Extraction, isolation and purification of constituents is bioassay guided, wherein the bioassays are the colorimetric Microplate Alamar Blue assays (MABA) done in collaboration with the Institute for TB Research, University of Illinois, Chicago, U.S.A. The pure compounds are identified through a combination of spectroscopic techniques.

Prior to the investigations that used *M. tuberculosis* H_{37}Rv for screening, Chua and co-workers [9] conducted a microbiological screening (agar cup method) of 125 plants for inhibitory activity against the acid-fast bacteria, *Mycobacterium* 607, together with representative Gram-positive (*S. aureus* and *B. subtilis*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*), and yeast (*S. cerevisiae* and *C. albicans*). This was succeeded by another study on 27 plants screened only for possible inhibition of *Mycobacterium* 607 [10]. As a result of both studies, priority plants were identified for in-depth phytochemical investigation guided by bioassays, still using *Mycobacterium* 607.

Initial studies using *M. tuberculosis* H_{37}Rv were based on the BACTEC 460 radiorespirometric assay. The results of bioassay guided phytochemical studies showed that there was little correlation between the inhibition of *Mycobacterium* 607 and the inhibition of *M. tuberculosis* H_{37}Rv. The samples (extracts, sub-extracts, fractions) which inhibited *Mycobacterium* 607 did not inhibit or were weakly inhibiting the growth of *M. tuberculosis* H_{37}Rv. A later study involving *M. tuberculosis* H_{37}Rv showed that the results of assays based on MABA were highly correlated with the results obtained from the BACTEC assay [11]. As a result, MABA became the method of choice for succeeding assays using *M. tuberculosis* H_{37}Rv.

This review paper covers studies published from 1999 to 2014 about natural products from Philippine medicinal plants which exhibited growth inhibitory activity *in vitro* against slow-growing, non-resistant strains of *M. tuberculosis* H_{37}Rv. Compounds with antitubercular properties at minimal inhibitory concentrations (MICs) of less than 128 μg/mL were highlighted and grouped according to their source of origin (i.e. plant family) and/or chemotypes. The selection includes natural products isolated mainly from plants, which were classified according to their secondary metabolite type as terpenes (triterpenes), steroids (sterols, sterones), alkaloids (indole, quinoline), aromatics (flavonoids, chalcones,

xanthones, chromones, etc.), and phenyldecanoids. In some cases, the collection also covers those structurally significant compounds with low inhibitory activity (MICs of >128 μg/mL). This review paper also highlights plant compounds that have been previously reported in literature, but which have been re-isolated and observed to exhibit anti-TB activity.

Antitubercular constituents of selected Annonaceae species

Efforts on the discovery of antimycobacterial compounds from the custard family, Annonaceae, have been mainly inspired by a number of studies that reported on the structural complexity and potent biological activity of natural products identified to date. Studies dating back as early as 2007 have pointed out discoveries relating to highly oxygenated cyclohexenes, tetrahydroxanthenoids, styryl-lactones, and alkaloids as antituberculosis agents.

***Goniothalamus gitingensis* Elmer.** Phytochemical studies enabled the isolation and identification of four secondary metabolites corresponding to three styryllactones, isoaltholactone (**1**), altholactone (**2**) and goniopypyrone (**3**), and the alkaloid liriodenine (**4**) from the Philippine endemic Annonaceae species, *Goniothalamus gitingensis*. The extracts together with the isolated compounds (**1-4**) exhibited good inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC up to 16 mg/mL). Liriodenine (**4**) showed the strongest antimycobacterial activity (MIC = 16 mg/mL) followed by **1** and 5:4 mixture of **1** and **2** [12].

***Uvaria valderramensis* Cabuang, Exconde & Alejandro.** The new Philippine endemic *Uvaria valderramensis* is a small shrub (ca. 3–7 m tall) growing in the lowlands and forests of Valderrama, Antique, Panay Island, Philippines, and is known locally as *usog* [13]. Its identification and distinction from other *Uvaria* species were facilitated by morphological and molecular phylogenetic evidences. Two new tetrahydroxanthene-1,3(2H)-dione metabolites, valderramenols A (**5**) and B (**6**), were isolated from this plant and were identified. Valderramenol A (**5**) showed antitubercular activity (MIC = 10 μg/mL), while grandiuvarone (**7**) and reticuline (**8**) had weaker activities (MIC = 32 μg/mL) [14].

***Uvaria rufa* Blume.** The Philippine medicinal plant *Uvaria rufa* (*susung kalabaw* in Filipino) is a short climbing shrub found in low- and medium-altitude forests of Northern Luzon, Philippines. In a previous report, the chloroform extract and fractions of *U. rufa* have been reported to exhibit strong antitubercular activity [15–16]. However, the major compounds such as (+)–zeylenol (**9**), ellipeiopsol B (**10**), ferrudiol (**11**), kweichowenol B (**12**), anabellamide (**13**), microcarpin A (**14**), and microcarpin B (**15**)

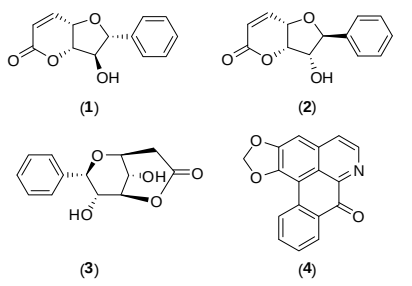

Figure 1. Secondary metabolites isolated from *G. gitingensis*

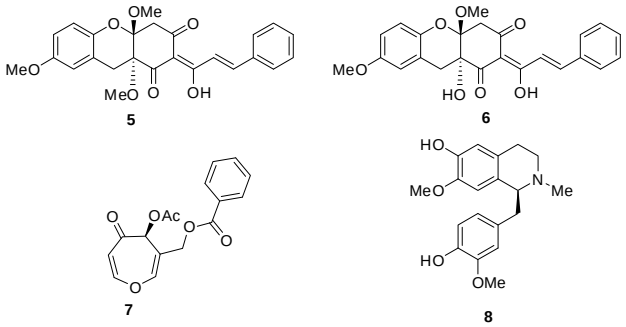

Figure 2. Compounds isolated from *U. valderramensis*

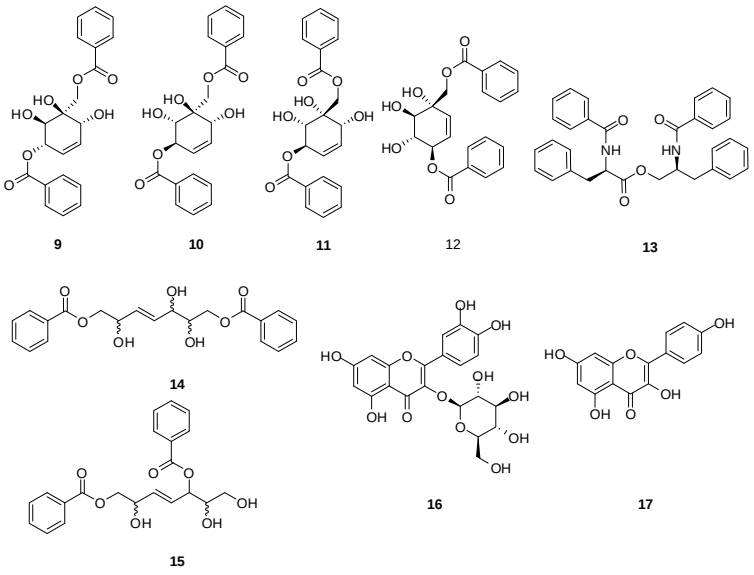

Figure 3. Compounds identified from *U. rufa*

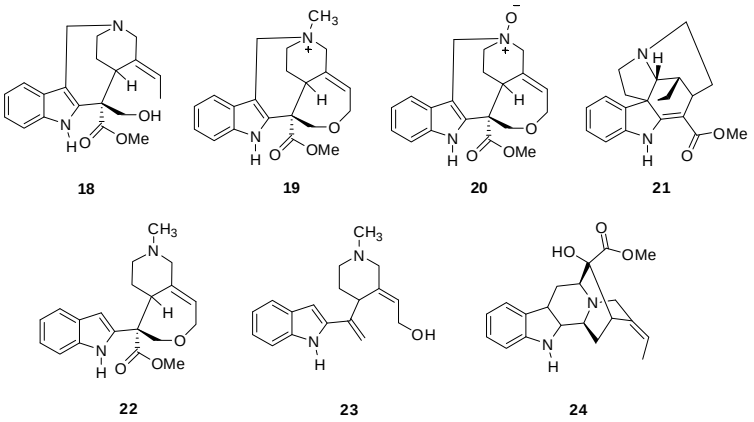

Figure 4. Indole alkaloids from *A. scholaris*

showed weak activity against *M. tuberculosis* (MIC >128 μg/mL). In another study, the flavonoids quercetin β-D-glucoside (**16**) and kaempferol (**17**) from the *n*-butanol extract exhibited moderately strong *in vitro* inhibitory activity against *M. tuberculosis* $H_{37}Rv$ (MIC = 64 μg/mL) [16].

ANTITUBERCULAR ALKALOIDS, STEROIDS, AND TERPENOIDS FROM SELECTED APOCYNACEAE SPECIES

The growing interest in exploring plants of the Apocynaceae family for antitubercular potential mainly spurred from studies reporting indole alkaloids with growth inhibitory effects against a panel of bacterial species. Thus, from 1999 up to the present, five species have been explored

enabling the identification of alkaloids, steroids, and terpenoids as antimycobacterial constituents.

***Alstonia scholaris* L. R. Brown.** *Alstonia scholaris* is a well-known Philippine medicinal plant, where the root bark is known for its antimalarial properties**.** The crude methanolic extract of *A. scholaris* collected in Manila demonstrated *in vitro* antituberculosis activity (89% inhibition against *M. tuberculosis* $H_{37}Rv$ at 50 μg/mL). Gradient pH fractionation of the alkaloids yielded three alkaloid extracts, AsA, AsB, and AsC, which exhibited 69%, 99%, and 99% inhibition, respectively. Group separation by silica gel vacuum liquid chromatography (VLC) of extracts AsA and AsB afforded fractions that showed 69–99% inhibition against the test mycobacterium. The previously reported indole alkaloids — 19,20*E*-vallesamine (**18**), a mixture of angustilobine B N_4-oxide (**19**) and N_4-methyl angustilobine B (**20**), 20*S*-tubotaiwine (**21**), 6,7-*seco*-angustilobine B (**22**) and (+)-manilamine (**23**) from the most bioactive alkaloid fractions with 98–99% inhibition — showed weak activities. Among the six compounds, only alkaloid 20*S*-tubotaiwine (**21**) demonstrated the highest activity with a minimum inhibitory concentration (MIC) of 100 μg/mL. Compared with the standard rifampin (MIC = 0.125 μg/mL), all alkaloids were considered inactive [17, 18]. In a separate study on *A. scholaris* collected in Ilocos Sur, fractions and alkaloid, including the major alkaloid, 19*E*-akuammidine (**24**) showed weak inhibition against *M. tuberculosis* $H_{37}Rv$ (MIC = >128 μg/mL) [19].

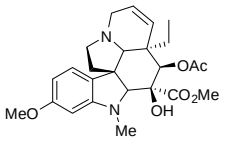

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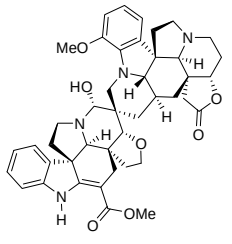
Figure 5. Structure of vindoline from *C. roseus*

***Catharanthus roseus* L. G. Don.** The Madagascar periwinkle, *Catharanthus roseus* is a common evergreen sub-shrub growing 1 m tall and occurs worldwide. Our study allowed the isolation and identification of the indole alkaloid vindoline (**25**) as the antimycobacterial constituent (MIC = 128 μg/mL) [20].



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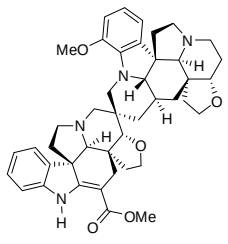

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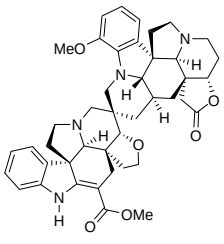

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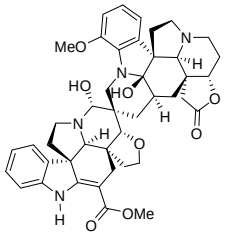

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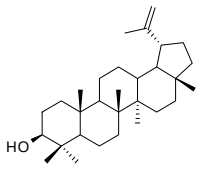

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Figure 6. Bisindole alkaloids and a triterpenoid from *V. globosa*

***Voacanga globosa* Merr.** *Voacanga globosa* is one of the two known endemic species of *Voacanga* in the Philippines and is used traditionally to stupefy eels and treat ulcers and wounds [21]. Globospiramine (**26**), a new spirobisindole alkaloid possessing an Aspidosperma-Aspidosperma skeleton, together with deoxyvobtusine (**27**), deoxyvobtusine lactone (**28**), vobtusine lactone (**29**), and lupeol (**30**), were isolated and identified through a bioassay-guided purification. Globospiramine (**26**) showed potent antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ as evidenced in MABA (MIC = 4 μg/mL) and low-oxygen recovery assays (LORA) (MIC = 5.2 mg/mL) [22].

***Voacanga megacarpa* Merr.** *Voacanga megacarpa* is one of the two endemic species found in the lowland forests of Camarines Sur province. Chromatographic purification of the crude DCM-methanolic extract afforded a 1:1 mixture of lupeol acetate (**31**) and β-amyrin acetate (**32**), and a 1:1 mixture of stigmasterol (**33**) and β-sitosterol (**34**). The crude DCM-methanolic and alkaloid extracts, and fractions (1 and 2) showed moderate inhibitory activity against *M. tuberculosis* $H_{37}Rv$ (MIC = 64 μg/mL) [23].

Antitubercular constituents from selected Rubiaceae species

A family distinguished for its economic and medicinal impacts, the Rubiaceae has been tapped to discover antitubercular constituents and other bioactives. Phytochemical efforts thus afforded triterpenes, and sterols as bioactive chemical ingredients.

***Morinda citrifolia* Linn.** *Morinda citrifolia* is a well-known plant in the Indo-Pacific region and grows everywhere in the Philippines. Popularly known as "noni," the plant has many uses in traditional medicine. For example, it is used as a treatment for dysentery, heartburn, liver disease and as an antidiabetic [24, 25]. The crude extract and hexane fractions from *M. citrifolia* showed 89% and 95% inhibition, respectively, against *M. tuberculosis* at 100 μg/mL. When tested against *M. tuberculosis*, *E*-phytol (**35**) and cycloartenol (**36**) exhibited MICs of 32 μg/mL and 64 μg/mL, respectively. The sterols stigmasterol (**33**) and β-sitosterol

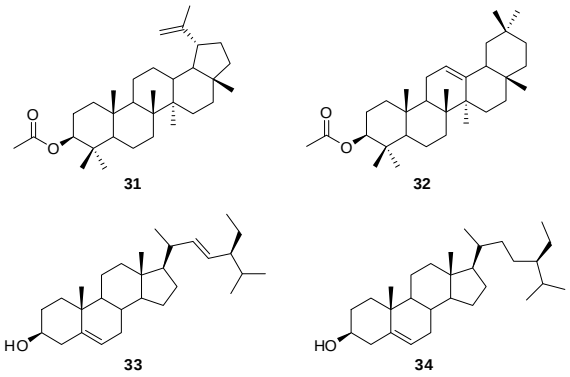


Figure 7. Sterols and triterpenes from *V. megacarpa*

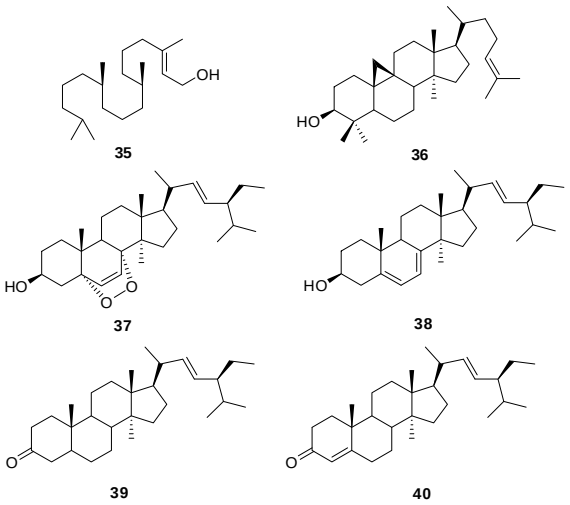


Figure 8. Sterols and triterpenes from *M. citrifolia*

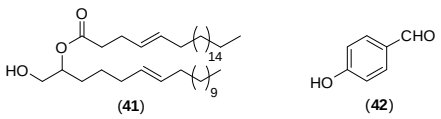


Figure 9. Compounds from *V. odorata*

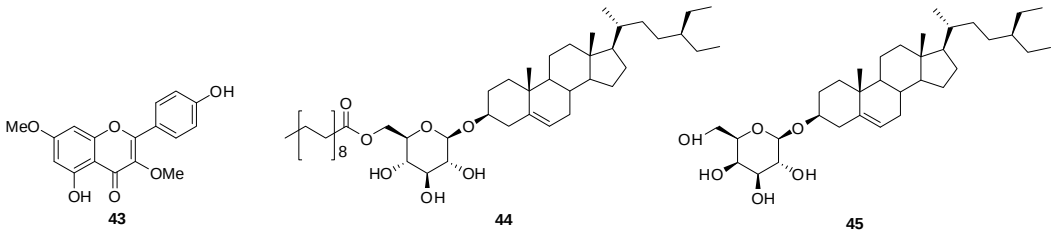
(**34**), and the epidioxysterol (**37**) (from oxidation of **38**) exhibited MICs of 128 μg/mL, 32 μg/mL and 2.5 μg/mL, respectively, while a 2:1 mixture of the ketosteroids stigmasta-4-en-3-one (**39**) and stigmasta-4,22-dien-3-one (**40**) showed an MIC of 2.0 μg/mL. There was insufficient material of **39** and **40** to test these compounds individually [26].

***Villaria odorata* (Blanco) Merr.** *Villaria odorata* is a flowering and fruiting shrub or small tree that is distributed in Luzon (Bulacan, Quezon, Albay, Cagayan, Isabela, Camarines, and Sorsogon Provinces), Visayas (Leyte) and Mindanao (Surigao Province). Villarinol (**41**), a new alkenoyloxy alkenol metabolite was isolated from the dichloromethane extract along with the known compounds stigmasterol (**33**) and 4-hydroxybenzaldehyde (**42**). The extracts and compounds of *V. odorata* showed weak inhibition against *M. tuberculosis* $H_{37}Rv$ (MIC >128 μg/mL) [27].

Antitubercular constituents from selected Zingiberaceae species

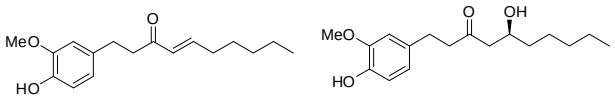
Better known as the ginger family, the Zingiberaceae is mainly tropical in distribution, and used for its spices, perfume, ornamental and medicinal properties. Interest in species of this family developed from significant findings regarding the inhibitory activity of ginger. Phytochemical work for antitubercular constituents gave phenyldecanoids, sterols and their glycosides and a flavonoid.

***Alpinia purpurata* (Vieill.) K. Schum.** *Alpinia purpurata* is locally known in the Philippines as *luyang pula* or red ginger, and is a native to the Pacific. MABA assay result of its crude ethanolic extract obtained from various plant parts had shown the leaf extract to possess the highest activity followed by the rhizome and flower extracts. Among the sub-extracts, the DCM sub-extract exhibited the highest activity followed by hexane and n-butanol sub-extracts. All fractions obtained from the hexane and DCM sub-extracts showed low to moderate activity. Fractionation and purification afforded a mixture of C_{28} to C_{32} fatty alcohols, a 3-methoxyflavone and two steroidal glycosides. The two latter metabolites were spectroscopically identified as kumatakenin (**43**), sitosteryl-3-*O*-6-palmitoyl-β-D-glucoside (**44**), and β-sitosteryl galactoside (**45**). The fatty alcohols showed an MIC value of 64 μg/mL and proved most active compared to the flavonoid kumatakenin and the steroidal glycosides (MIC >128 μg/mL) [28].



43 **44** **45**

Figure 10. Sterol glycosides and a flavonoid from *A. purpurata*



46 **47**

Figure 11. Phenyldecanoids from *Z. officinale*

***Zingiber officinale* Rosc.** The widely occurring food ingredient, *Zingiber officinale* has been traditionally used for arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases, and helminthiasis. *In vitro* anti-TB susceptibility assay using the radiorespirometric method showed that a crude alcoholic extract of the rhizome of *Z. officinale* inhibited the growth of *M. tuberculosis* $H_{37}Rv$ by 100% at 1000 μg/mL. Bioassay-guided isolation of the constituents present in the bioactive hexane fraction afforded two phenyldecanoids namely, 6-shogaol (**46**) and 6-gingerol (**47**). Compounds **46** and **47** exhibited 100% inhibitory activity at 64 and 32 μg/mL, respectively [29].

Antitubercular constituents from miscellaneous plant species

***Abutilon indicum* Sweet.** *Abutilon indicum* (Malvaceae) is used in Filipino folk medicine as demulcent, diuretic, sedative, aphrodisiac and antidiabetic remedy [30]. The fractions obtained after silica gel chromatography of the crude DCM-methanol (1:1) showed the first fraction (MIC = 64 μg/mL) to exert the highest inhibition against *M. tuberculosis* $H_{37}Rv$. Further separation of this fraction afforded sub-fractions with moderately strong inhibitory activity against the test organism (MIC up to 64 μg/mL). Chromatographic purification of sub-fraction 1 afforded four compounds which were spectroscopically identified as β-amyrin 3-palmitate (**48**), squalene (**49**) and a 1:1 mixture of the sterols β-sitosterol (**33**) and stigmasterol (**34**). Evaluation of the antimycobacterial activity of **33**, **34**, **48**, and **49** showed insignificant inhibitory activity against the test organism (MIC >128 μg/mL) [31].

***Lunasia amara* Blanco.** *Lunasia amara* (Rutaceae) is an erect shrub that grows in thickets and forests throughout the Philippines [32]. An ethanolic leaf alcohol extract showed significant inhibition to *M. tuberculosis* $H_{37}Rv$ (99% at 1000 μg/mL). Bioassay-guided chromatographic purification of the hexane extract (99% at 100 μg/mL) and dichloromethane extract (93% at 100 μg/mL) afforded two quinoline alkaloids which were identified spectroscopically as kokusagine (7,8-methylenedioxydictamnine) (**51**) and 4-methoxy-2-phenylquinoline (**52**). Isolation of alkaloids from the pH 4 extract of another batch of ethanolic extract gave graveolinine (4-methoxy-2-(3'4'-methylenedioxy)phenylquinoline) (**53**). Alkaloids **51** and **53** demonstrated an MIC of 16 μg/mL while **52** exhibited an MIC of 30 μg/mL [33].

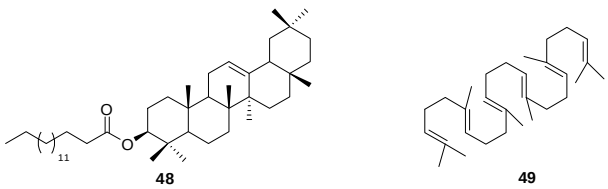


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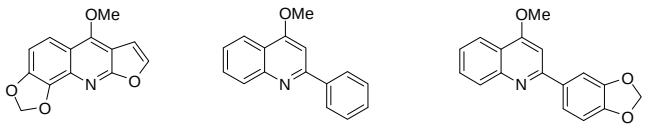
Figure 12. Triterpenes from *A. indicum*



51 52 53

Figure 13. Quinoline alkaloids from *L. amara*

***Momordica charantia* L.** *Momordica charantia* (Cucurbitaceae) or *ampalaya* in Filipino is one of the most popular anti-diabetic plants in Asia, Africa and Latin America and is traditionally used as food and medicine [34]. A new lanostane aldehyde, charantal (**54**), was isolated from the ethanolic (leaf) extract together with the known compound, 2,4-bis(2-phenylpropan-2-yl)phenol (**55**). Compound **55** displayed a moderately strong antitubercular activity against *M. tuberculosis* $H_{37}Rv$ (MIC = 14 μg/mL) [35].

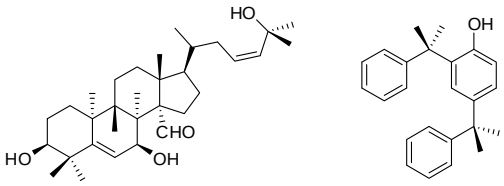


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Figure 14. Compounds from *M. charantia*

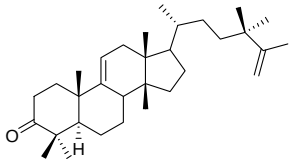

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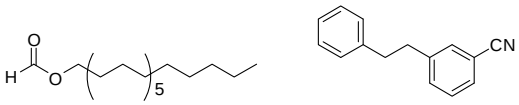
Figure 15. Structure of 24,24-dimethyl-5b-tirucall-9(11),25-dien-3-one from *P. laevis*

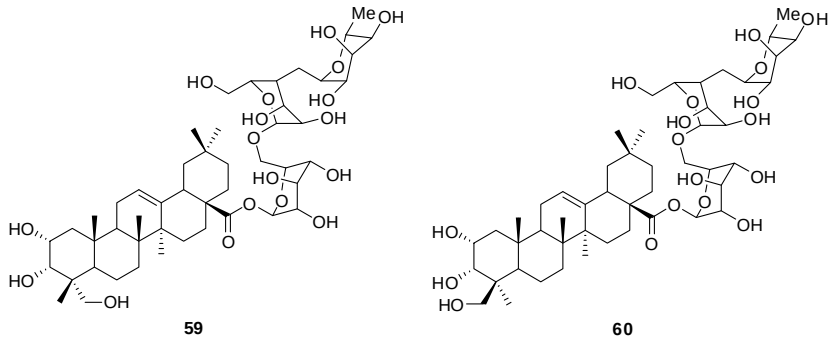


57 58

Figure 16. Compounds from *P. odorata*

***Pandanus tectorius* Soland. var. laevis.** *Pandanus tectorius* Soland. var. laevis (Pandanaceae**)** is known for the use of its dried roots as diuretic. During the bioassay-guided purification of the antitubercular chloroform extract of *P. tectorius* leaves a new tirucallane-type triterpene, 24,24-dimethyl-5b-tirucall-9(11),25-dien-3-one (**56**) was afforded along with squalene (**49**) and a mixture of the phytosterols, β-sitosterol (**33**) and stigmasterol (**34**). MABA showed that **56** inhibited the growth of *M. tuberculosis* $H_{37}Rv$ with an MIC of 64 μg/mL, while squalene (**49**) and the sterol mixture have MICs of 100 and 128 μg/mL, respectively [36].

***Premna odorata* Blanco.** *Premna odorata* (Lamiaceae) is a medicinal plant traditionally used in Albay Province, Philippines to treat tuberculosis. The crude methanolic extract and sub-extracts showed poor inhibitory activity against *M. tuberculosis* $H_{37}Rv$ (MIC >128 μg/mL). However, increased inhibitory potency was observed for fractions eluted from the DCM sub-extract (MIC = 54–120 μg/mL). Further purification of the most active fraction (MIC = 54 μg/mL) led to the isolation of a 5:3 mixture of



59 60

Figure 17. Triterpene glycosides from *S. odorata*

1-heneicosyl formate (**57**) and 3-(2-phenylethyl)benzonitrile (**58**), and 4:1 mixture of β-sitosterol (**33**) and stigmasterol (**44**) which were identified through GC-MS analysis (with dereplication) and NMR experiments. The MIC of the mixture of compounds **57** and **58** was 8 μg/mL [37].

***Schefflera luzoniensis* Merr.** *Schefflera luzoniensis* (Araliaceae) is an endemic small shrub abundant in the lowland to medium forests of Mount Banahaw, Southern Luzon, Philippines. The antibacterial potential of Schefflera luzoniensis was assessed using the paper disc diffusion and MABA assays. Moderately strong zones of inhibition were observed for the n-butanol subextract and the saponin-rich fractions against the Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Also, the saponin-containing subfractions showed activity against M. tuberculosis H37Rv (MIC=64 μg/mL). HPLC-MS profiling of the antibacterial fractions revealed the presence of two known oleanene glycosides, namely, schiffoleoside A (**59**) and F (**60**) as the major saponin glycoside constituents. Angiogenic property of the n-butanol subextract was revealed by the chicken chorio-allantoic membrane assay at a concentration of 10 μg/mL while higher concentrations suppressed neovascularization (**58**).

Conclusion

The present review compiled 60 natural products isolated and identified from a number of Philippine plants. Those molecules with MICs less than 128 mg/mL would be considered as potential leads for further investigations in the discovery and development of more potent antitubercular congeners. For example, valderramenol A (**5**), globospiramine (**26**), kokusagine (**51**), 4-methoxyphenylquinoline (**52**), and 2,4-bis(2-phenylpropan-2-yl)phenol (**55**) are remarkable scaffolds for drug development, because of their potent antimycobacterial activity. It is also worth noting the synergistic antitubercular activity of compound mixtures, for example, of stigmasta-4-en-3-one (**39**) and stigmasta-4,22-dien-3-one (**40**) and of 1-heneicosyl formate (**57**) and 3-(2-phenylethyl)benzonitrile (**58**). Current efforts are centered on investigations of other Philippine medicinal plant families. Similar investigations are also ongoing on antitubercular constituents from microbial sources such as endophytic fungi.

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