REVIEW

A review of studies on bioactive compounds isolated from Sri Lankan flora

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Summary: The biodiversity of Sri Lanka based on the average number of plant species per 10,000 km^2 has been stated to be much higher than in any other country in Asia. Despite the diversity and the potential economic value of these plants, many have not been investigated for their natural products. Also both the endemic and non-endemic plants species, which have been investigated chemically and biologically show a wide variety of bioactive compounds indicating the high economic potential of the flora. This paper reviews the studies of bioactve compounds isolated from Sri Lankan flora during the period 1980 to date.

Keywords: Biological activity, endemic plant species, medicinally important compounds, Sri Lankan flora.

INTRODUCTION

The World Conservation Monitoring Centre has designated Sri Lanka as a 'hotspot' in terms of its biodiversity (Caldecott *et al.*, 1994). The indigenous flora of Sri Lanka comprises about 7,500 plant species. Of the recorded 3,154 flowering plant species, 894 (28 %) species are known to be endemic to the island (Wijesundara *et al.*, 2012). There are also records of 336 species of pteridophytes, which include 49 endemics (MOE, 2012), 650 species of lichens belonging to the family Thelotremataceae (41 endemics), 575 mosses (endemism unknown), 303 liverworts (endemism unknown), 1920 species of fungi and some 900 species of algae (Somesekaram *et al.*, 1997). The variety, richness and abundance of Sri Lankan flora and the high percentage of endemic plants - compared to most countries in the region - makes it an ideal location for a systematic drug discovery programme. The early botanical description of Sri Lankan plants is contained in a series of books (Trimen, 1885), which have been revised later (Dassanayake, 1996). Endemic plants in Sri Lanka are within easy access as more than 90 % of them are located in a small area of about 15,000 km^2 in the low country wet zone and the montane zone (Gunatilleke & Gunatilleke, 1990).

A large number of plants with medicinal value has been listed in a compilation, the 'Sinhalese Materia Medica' (Attygala, 1917). Information on the chemistry and the pharmacology of some Sri Lankan and Indian plants (Chandrasena, 1935) and medicinal plants used in Sri Lanka (Jayaweera, 1982) is also documented.

During the past 40 years there have been several investigations on the chemical constituents and antimicrobial and insecticidal effects of Sri Lankan plants, particularly the endemics (Bandara *et al.*, 1989a; 1989b; 1990b; Hewage *et al.*, 1997; 1998). In addition the biological activity of compounds isolated from Sri Lankan lichens have also been determined (Nanayakkara *et al.*, 2005). Tropical lichens, particularly those from Sri Lanka, continue to show a wide variety of biologically active compounds (Orange *et al.*, 2001; Karunaratne *et al.*, 2002; 2005; Jayalal *et al.*, 2012). Antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection has been reported by Munasinghe *et al.* (2001). Arseculeratne *et al.* (1985) have screened fifty medicinal plants for hepatotoxic activity. In a ground breaking study, the oral hypoglycaemic activity of some Sri Lankan plants have been reported by Karunanayake *et al.* (1984). The alkaloids found in

Sri Lankan plants and their biological activities have been reviewed by Gunatilaka (1999).

However, in many of the early studies on bioactive compounds, the activity determinations were restricted to antibacterial and mosquito larvicidal activities, which are too broad to be useful determinants of high value bioactivities such as, antioxidant, insecticidal, α-glucosidase, cytotoxic, enzyme inhibitory, spermicidal and anticancer activities. These compounds have become important and highly sought after. This review highlights the biological activity of compounds reported from endemic and non-endemic flora of Sri Lanka.

Antioxidant activity

Free radicals play an important role in carcinogenesis through their involvement in breaking the DNA strands. Free radicals also play a role in arteriosclerosis, malaria, in rheumatoid arthritis and could play a role in neurodegenerative diseases and the ageing process (Atta-ur-Rahman & Choudhary, 2001).

Free radicals are unique bioactive mediators, which account for the tonic relaxation of all types of blood vessels and nonadrenergic and noncholinergic relaxation of the gastro intestinal tract in low concentration. At high concentrations they can damage DNA, RNA, lipids and proteins leading to increased mutations and altered enzymes, which in turn lead to carcinogenesis (Halici *et al.*, 2005).

Xylopia championii Hook & Thomas (Annonaceae) is endemic to Sri Lanka. Out of the five alkaloids isolated from stem bark and stem, (-)-discretine (Figure 1a) and (+)-laudanidine (Figure 1b), have shown a high antioxidant activity at a concentration of 0.5 mg/mL, compared to the standard DL-α-tocopherol in the DPPH assay (Puvanendran *et al.*, 2008).
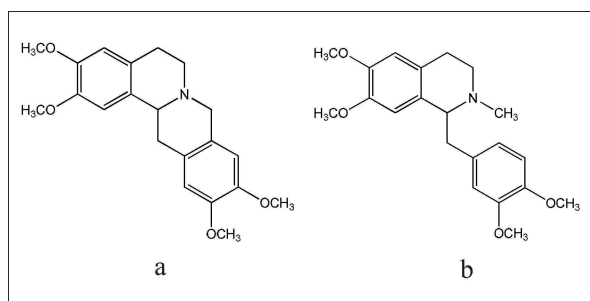
Chromatographic separation of the methanol extract of *Elaeocarpus serratus* L., leaves has afforded 3-O-L-rhamnopyranosyl-5,7-dihydroxy-2-(3,4,5-trihydroxy-phenyl)-chromen-4-one (myricitrin) (Figure 2). The antioxidant activity of this compound has been evaluated by the DPPH radical-TLC bio-autography method where it has shown strong off-white spot on the TLC plate at 0.1 µg/spot indicating antioxidant activity comparable to standard ascorbic acid (IC_{50} = 3.9 µg/mL) and butylated hydroxylanisole (IC_{50} = 3.6 µg/mL) (Jayasinghe *et al.*, 2012).
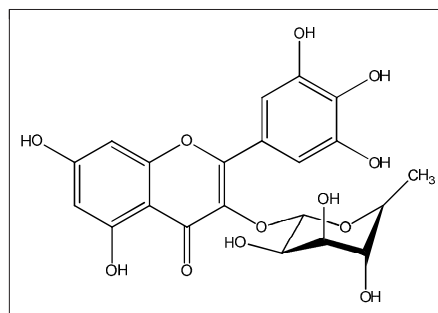

Figure 2: Structure of myricitrin

The two stilbene derivatives, (E)-4-isopentenyl-3,5,20,40-tetrahydroxystilbene (Figure 3a) and (E)-4-(3-methyl-E-but-1-enyl)-3,5,20,40-tetrahydroxystilbene (Figure 3b) isolated from the methanol extract of the stem bark of *Artocarpus nobilis* Thwaites have shown a significant antioxidant activity towards the DPPH radical in the TLC bio-autography method at 1 µg/spot (Jayasinghe *et al.*, 2004).

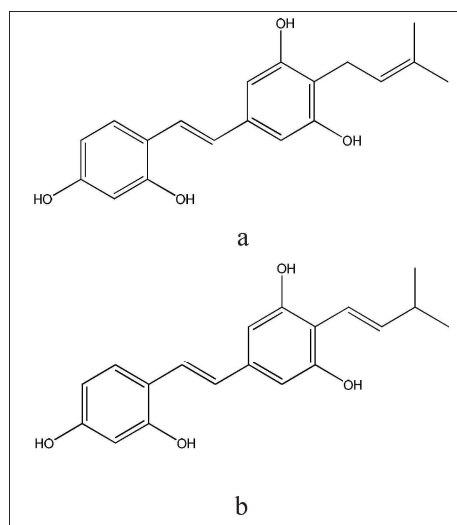**Figure 1:** Structure of (a) (-)-discretine, (b) (+)-laudanidine

Figure 3: Structure of (a) (E)-4-isopentenyl-3,5,20,40-tetrahydroxys-tilbene and (b) (E)-4-(3-methyl-E-but-1-enyl)-3,5,20,40-tetrahydroxystilbene

Chemical investigation of the dichloromethane extract of the leaves of *Syzygium jambos* L., have resulted in three dihydrochalcones, phloretin (4'-O-methyl ether (2',6'-dihydroxy-4'-methoxydihydrochalcone) (Figure 4a), myrigalone G (2',6'-dihydroxy-4'-methoxy-3'-methyldihydrochalcone) (Figure 4b), and myrigalone B (2',6'-dihydroxy-4'-methoxy-3,5'-dimethyldihydrochalcone) (Figure 4c) with radical scavenging properties towards the DPPH radical (Jayasinghe *et al.*, 2007).
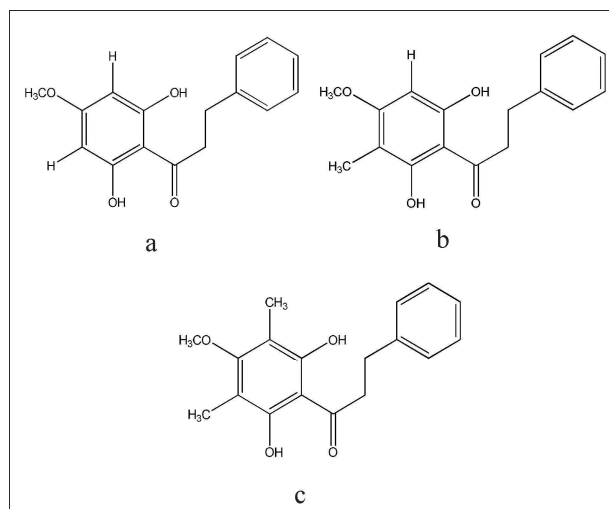

Figure 4: Structure of (a) phloretin; (b) myrigalone G and (c) myrigalone B

Among the three compounds isolated, myrigalone B has exhibited the highest free radical scavenging activity with an IC_{50} value of 3.8 µg/mL compared with that of the standard DL α-tocopherol (IC_{50} = 7.3 µg/mL). Myrigalone G has shown moderate

free radical scavenging activity (IC_{50} = 10.6 µg/mL), while phloretin has exhibited low antioxidant activity (IC_{50} = 30 µg/mL) compared to that of DL α-tocopherol (Jayasinghe *et al.*, 2007).

Insecticidal activity

From the early Roman times to the mid 20th Century pyrethrum, rotenone, nicotine, sabadilla and quassin have been used widely as insect repellents and toxins. The indiscriminate use of synthetic insecticidal compounds has led to deterioration of the environment and they also induce the build up of resistant pest populations, eliminate their natural enemies and facilitate the resultant development of secondary pests. Plants contain a wide diversity of secondary metabolites, some of which play an important role in their defense against pathogens and herbivores (Ware & Whitaker, 2004). Botanicals used as insecticides presently constitute 1 % of the world insecticide market (Rozman *et al.*, 2007).

Pyxine consocians is a lichen growing on the stem bark of the palm tree *Roystonia regia*. The hexane extract of *P. consocians* has led to the isolation of three mosquito larvicidal compounds against *Aedes aegypti* at 10 ppm: cabraleadiol monoacetate (Figure 5a) (90 % moribund after 24 h), 4-*O*-methylcryptochlorophaeic acid (Figure 5b) (60 % dead after 24 h) and lichexanthone (Figure 5c) (80 % moribund after 24 h) (Kathirgamanathar *et al.*, 2006).

The investigation of biologically active constituents of *Heterodermia leucomelos*, a lichen collected from the mossy rocks in the Horton Plains National Park, Nuwara Eliya District, Sri lanka, has led to the isolation of mosquito larvicidal active compound, 3,6-dimethyl-
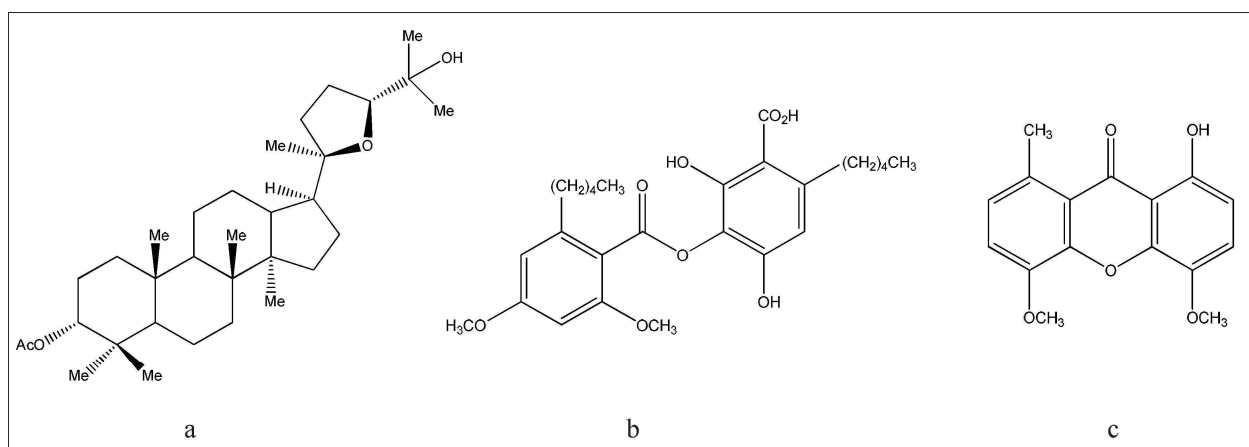

Figure 5: Structure of (a) cabraleadiolmonoacetate; (b) 4-*O*-methylcryptochlorophaeic acid and (c) lichexanthone

2-hydroxy-4-methoxybenzoic acid (Figure 6) (90 % moribund after 24 h at 10 ppm) (Kathirgamanathar *et al.*, 2006). Hydroxy benzoic acids are important iron chelators (siderophores), which are secreted by some pathogenic bacteria that grow under low iron conditions (Feistner & Beaman, 1987; Karunaratne *et al.*, 1992). It is known that polar lichen substances are secreted out into the growing medium of the lichens, leading to the intriguing hypothesis that such bioactivities may form part of the chemical defense mechanisms of lichens (Karunaratne *et al.*, 2005).

The two butenolides (Figure 7a) and (Figure 7b) isolated from the CH_2Cl_2 extracts of three representative species of the endemic genus *Hortonia*, *H. floribunda*, *H. angustifolia* and *H. ovalifolia* have exhibited potent mosquito larvicidal activity against the 2nd instar larvae of *A. aegypti* with IC_{50} values of 0.41 and 0.47 ppm, respectively, highlighting the importance and the potency of five membered ring compounds (Ratnayake *et al.*, 2001; Piers & Karunaratne, 1983).
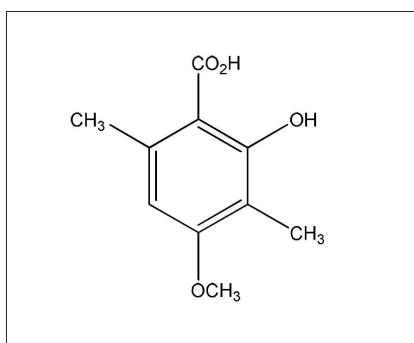

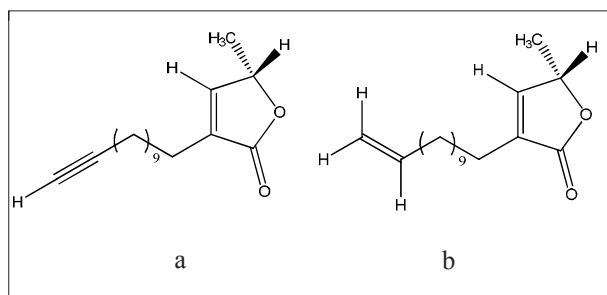**Figure 6:**   Structure of 3,6-dimethyl-2-hydroxy -4-methoxybenzoic acid

Figure 7:   Structure of two butenolides [(a) and (b)] from genus *Hortonia*

Activity guided isolation from the combined methanol and dichloromethane extracts of the stem bark of *Microcos paniculata* L., has provided an insecticidal
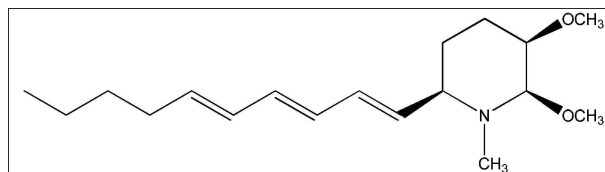

Figure 8:   Structure of n-methyl-6b-(decal-3',5'-trienyl)-3b-methoxy-2b-methylpiperidine
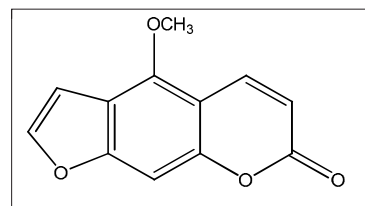

Figure 9:   Structure of bergapten

alkaloid, N-methyl-6b-(decal-3',5'-trienyl)-3b-methoxy-2b-methylpiperidine (Figure 8), which exhibited MC_{50} of 1.0 ppm and LC_{50} of 2.1 ppm at 24 h against *A. aegypti* second instar larvae. The MC_{50} and LC_{50} of the compound at 24, 48 and 72 h has indicated that a very few of the moribund larvae survived, most dying after about 72 h after treatment (Bandara *et al.*, 2000).

Bergapten (Figure 9) isolated from the stem bark of *Limonia acidissima* L., (Rutaceae), a medicinal plant widely distributed in Sri Lanka has tested positive for insecticidal activity against *Callosobruchus chinensis* L. Bergapten has reduced the emergence of adults by 100 % at 0.1 mg level (Bandara *et al.*, 1989b; 1990b).

The shot-hole borer, *Xyleborus fornicatus* Eichh., is a small, wood boring ambrosia beetle and is considered to be the most serious pest of Tea (*Camellia sinensis* var. *assimica*) in Sri Lanka. (+)-Usnic acid (Figure 10) has been isolated from an *Usnea* sp. collected from the surface of a rotting *Acacia decurrens* Willd., tree in Ambewela, Nuwara Eliya District, Sri Lanka. Healthy adult females of *X. fornicatus* exposed to different
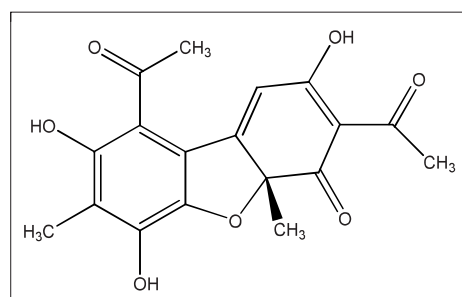

Figure 10: Structure of (+)-usnic acid

concentrations of (+)-usnic acid have shown an effect on the growth development of beetles. At 75 and 100 ppm, only 10 and 4 mother beetles have produced progeny, respectively. The galleries constructed in the test media containing (+)-usnic acid have been observed to be of an irregular shape compared to the control (Sahib *et al.*, 2008).

Along with (+)-usnic acid, two β-orcinol depsidone lactones, the methyl ethers of menegazziaic acid and stictic acid were isolated, in addition to glyceryl trilinolate. Usnic acid has exhibited potent antitermite activity against a common tea pest, *Glyptotermes dilatatus* at low elevations (Kathirgamanathar *et al.*, 2005).

The hot petrol extracts of the roots of *Croton lacciferus* L., has shown significant insecticidal activity against *Aphis cracciuora* Koch. The extract, when chromatographed over silica gel and eluted with petrol-chloroform has yielded three ent-kauranoids, two of which are new natural products. The two new kauranoids (Figure 11a & b) have been tested using the microapplicator method for insecticidal activity against *A. cracciuora* maintained in the laboratory on one-week-old potted cowpea, *Vigna unguiculata* L. These two compounds have shown a moderate insecticidal activity against *A. craccivora* at a dose of 5 ppm/insect causing 61 and 62 % mortality, respectively, of adult female aphids after 24 h (Bandara *et al.*, 1988a).
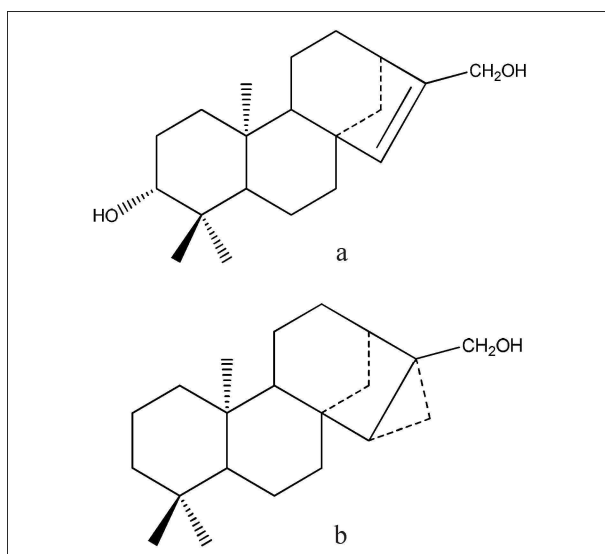
Diploclisia glaucescens (Blume) Diels (Memspermaceae) is a creeper growing in the mid-country regions of South India and Sri Lanka. Ecdysterone (Figure 12) along with a bidesmosidic triterpenoid saponin have been isolated from the methanol extract of the stem of *D. glaucescens* in a yield of over 0.46 %. It possessed insecticidal activity against groundnut aphid, *Aphis craccivora* with an LD_{50} value of 18 mg/kg (Bandara *et al.*, 1989c; d).

Croton aromaticus L., (Euphorbiaceae), a widely distributed plant in Sri Lanka, is used in ethnomedical preparations and in traditional agriculture. The air-dried and powdered roots of *C. aromaticus* have been subjected to sequential solvent extraction. The petroleum ether extract was chromatographed over silica gel when (-)-hardwickiic acid (Figure 13) was isolated in 0.8 %. The aphidicidal activity of the compound has been evaluated using the microapplicator method. The compound at a dose of 5 ppm/insect has caused 62 % mortality of adult female aphids after 24 h (Bandara *et al.*, 1987).
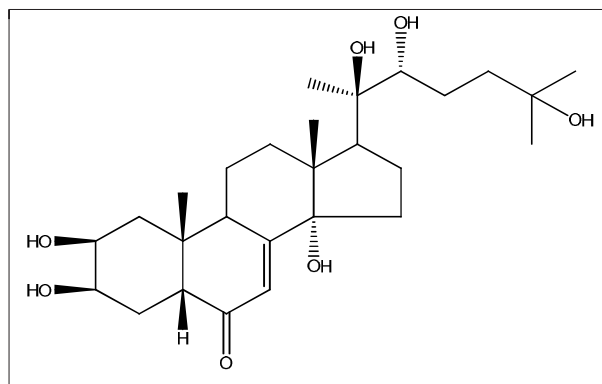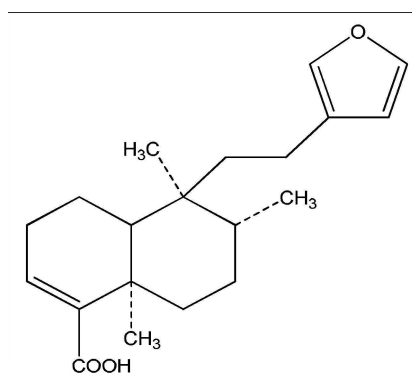

Figure 12: Structure of ecdysterone

Figure 11: Structure of two new kauranoids [(a) and (b)] from *Croton lacciferus* L.

Figure 13: Structure of (-)-hardwickiic acid

Antibacterial activity

The search for new antibiotics with hitherto unknown modes of action remains an urgent priority as microbial resistance to antimicrobial agents continues to unfold as one of the most difficult problems facing the physicians dealing with infectious diseases (Ristuccia & Cunha, 1984). Bacteria are capable of passing genetic information between species through a process called conjugation. This property and the relatively short generation time have been important and led to the current situation in which the control of pathogenic bacteria has been difficult. In 1961, outbreaks of methicillin resistant *Staphyloccocus aureus* (MRSA) were sporadic and the introduction of new antibiotics kept the bacteria at bay. Since then, MRSA has acquired resistance to virtually all antibiotics in clinical use including cephalosporins, tetracyclines, aminoglycosides, erythromycin and the sulfonamides (Neu, 1992).

Four lichen species, *Parmotrema grayana* Hue, *Cladonia* sp., *Heterodermia obscurata* (Nyl.), and *Roccella montagnei* Bel, collected from different locations in Sri Lanka have been investigated: *P. grayana* from the stem bark of palm tree [*Roystonea regia* (Kunth) O.F. Cook (syn. *R. elata*, *R. floridana*)]; *Cladonia* sp., from rocks in the Labukelle region, Central Province; *H. obscurata* from the rocks in the Beragala region, Central Province and *R. montagnei* from coconut palm trees (*Cocos nucifera* L.). Atranorin (Figure 14) isolated from all four lichen species has exhibited a moderate antibacterial activity against *Escherichia coli, Bacillus subtilis* and *Salmonella typhi* at a concentration of 100 µg/mL in the agar well diffusion method with inhibition zones of 13, 12 and 12 mm, respectively.

Sekikaic acid (Figure 15) isolated from *H. obscurata* has shown a moderate antibacterial activity against *E.coli, B. subtilis* and *S. typhi* at a concentration of 100 µg/mL in agar well diffusion method with an inhibition zone of 16 mm.

Methanolyis of lecanoric acid isolated from *Cladonia* sp., *H. obscurata* and *P. grayana*, has yielded the mononuclear aromatic compound methyl orsellinate (Figure 16), which exhibited a moderate antibacterial activity against *E. coli*, *B. subtillus* and *S. typhi* (Thadani *et al.*, 2012).

The methanol extract of the brown seaweed, *Stoechospermum marginatum* C. Agardh, grown in seas around the Northern part of Sri Lanka has been found to inhibit the growth of *Staphylococcus aureus* in the standard disk method. Fractionation of the active extract has led to the isolation of an antibacterial compound whose structure has been established to be 19-acetoxy-5,15,18-trihydroxyspata-13,16-diene (Figure 17) (De Silva *et al.*, 1982).
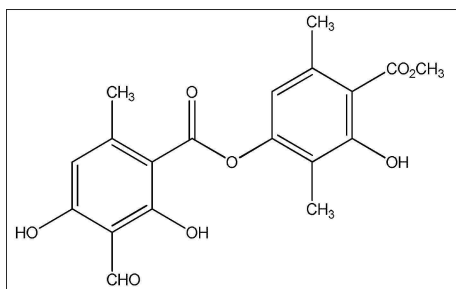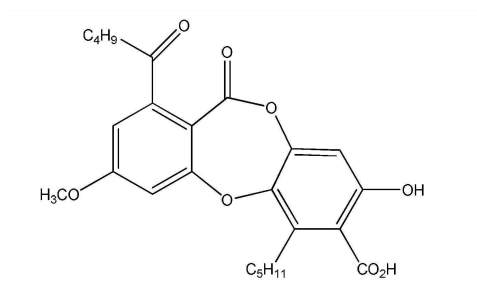

Figure 14: Structure of atranorin

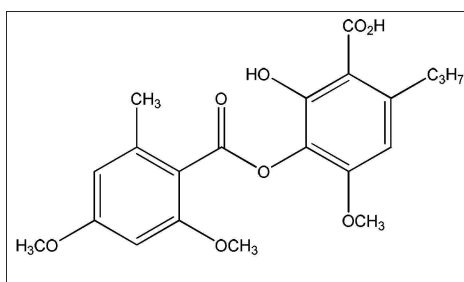**Figure 16:** Structure of methyl orsellinate

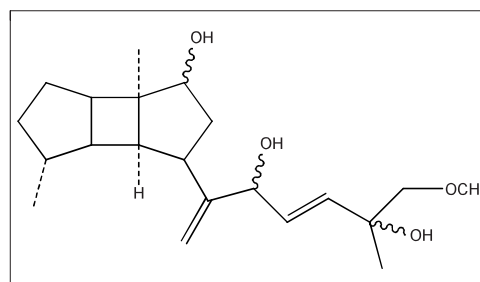**Figure 15:** Structure of sekikaic acid

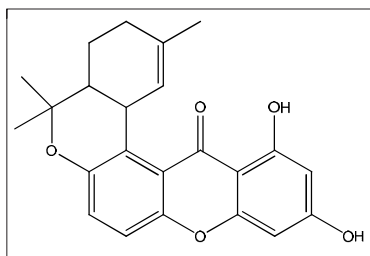**Figure 17:** Structure of 19-acetoxy-5,15,18-trihydroxyspata-13,16-diene
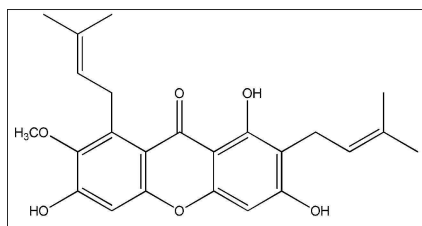
Figure 18: Structure of calozeyloxanthone

Figure 19: Structure of α-mangostin

Calozeyloxanthone (Figure 18), isolated from *Calophyllum thwaitesii* Planchon & Triana and *C. moonii* Wight, has shown a significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* giving a MIC value of 4.1 – 8.1 µg/mL, which is comparable with that of standard compounds, vancomycin (MIC = 0.5 – 4 µg/mL) and gentamycin (MIC = 0.5 – 1 µg/mL) (Dharmaratne *et al.*, 1999).

α-Mangostin (Figure 19), isolated from the stem bark of *Garcinia mangostana* L., has been found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) with MIC values of 6.25 and 6.25 to 12.5 µg/mL, respectively. Further, synergism between α-mangostin and gentamicin (GM) against VRE, and α-mangostin and vancomycin hydrochloride (VCM) against MRSA has been observed. Moreover, partial synergism between α-mangostin and commercially available antibiotics such as ampicillin and minocycline has also been discovered.
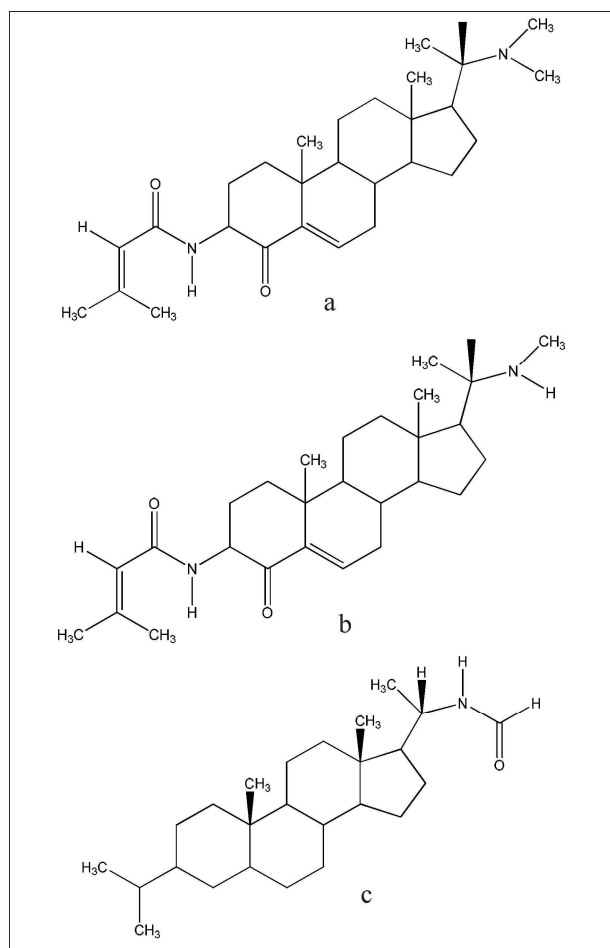
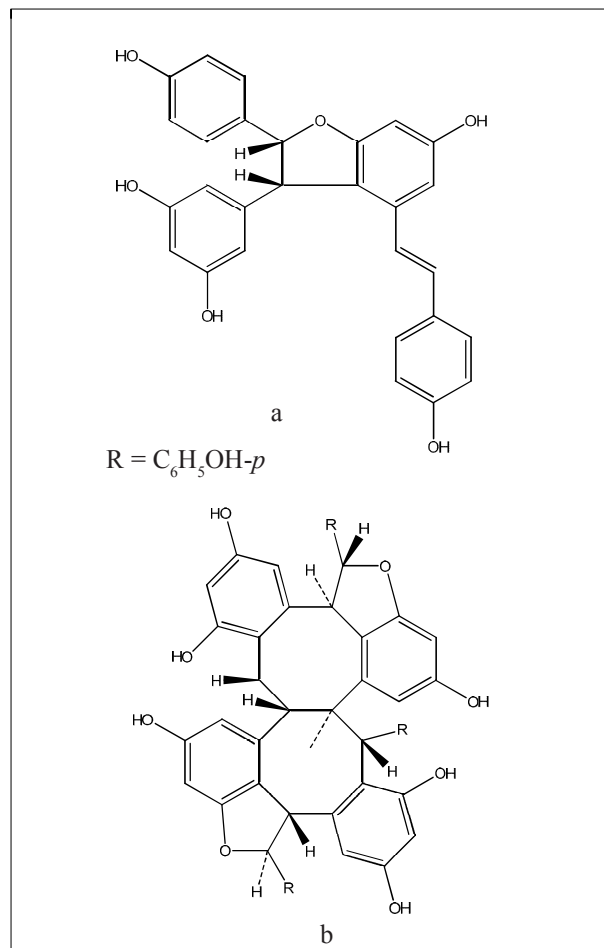

Figure 20: Structure of (a) epipachysamine-E-5-ene-4-on; (b) N_b-demethylepipachysamine-E-5-ene-4-on and (c) iso-N-formylchonemorphin

R = C_6H_5OH-*p*

Figure 21: Structure of (a) ε-viniferin and (b) vaticaffinol

These findings suggested that α-mangostin alone or in combination with GM against VRE and in combination with VCM against MRSA might be useful in controlling VRE and MRSA infections (Sakagami *et al.*, 2005).

The dried whole plant of *Sarcococca brevifolia* Muell., extracted with methanol and separated on silica gel yielded three steroidal alkaloids; epipachysamine-E-5-ene-4-on (Figure 20a), N_b-demethylepipachysamine-E-5-ene-4-on (Figure 20b) and *iso*-N-formylchonemorphin (Figure 20c). The evaluation of antibacterial activity by agar well diffusion method has indicated that epipachysamine-E-5-ene-4-on and *iso*-N-formylchonemorphin exhibit strong activity against a number of pathogenic bacteria. Antibacterial activity of epipachysamine-E-5-ene-4-on has been observed against *Bacillus cereus* (MIC = 0.0625 mg/mL), *Klebsiella pneumoniae* (MIC = 0.25 mg/mL) and *Staphylococcus aureus* (MIC = 0.03125 mg/mL). Similarly, *iso*-N-formylchonemorphin has shown antibacterial strong activity against *Bacillus cereus* (MIC = 0.1250 mg/mL), *Pseudomonas aeruginosa* (MIC = 0.1250 mg/mL) and *Staphylococcus aureus* (MIC = 0.0312 mg/mL) (Jayasinghe *et al.*, 1998).

Vatica affinis Thw., is a dipterocarp species endemic to Sri Lanka. The cold acetone extracts of the bark and timber of this species have been chemically investigated and a resveratrol dimer, ε-viniferin (Figure 21a), has been isolated from the extract by using silica gel preparative plates. A silica column chromatography of the extract has yielded a second compound vaticaffinol (Figure 21b), when the column was eluted with acetone-benzene (7:13). Both ε-viniferin and vaticaffinol isolated in this study has shown antibacterial activity towards Oxford *Staphylococcus* and *Escherichia coli* when tested by the filter paper disc method in Mueller Hinton Agar medium (Sotheeswaran *et al.*, 1985).

Calophyllum moonii Wight., is an endemic species to Sri Lanka. Calozeyloxanthone (Figure 22) has been re-isolated from the root bark of *C. moonii* and was tested for antibacterial activity against vancomycin-resistant *Enterococci* (VRE) and vancomycin-sensitive *Enterococci* (VSE). Results of the study has indicated that calozeyloxanthone is strongly active against both tested species with MIC values of 6.25 and 12.5 µg/mL, respectively. Further, a marked synergism between calozeyloxanthone and vancomycin hydrochloride (VCM) against VRE has been observed (Sakagami *et al.*, 2002).

Litsea gardneri L., is a moderate sized endemic plant in Sri Lanka. Alkaloids extracted from the defatted bark of *L. gardneni* have been tested for antibacterial activity against *E. coli* and *S. aureus* using the modified tube dilution technique. Four alkaloids have been isolated and the activity was observed only for laurolitsine (Figure 23) (against *S. aureus*), the minimum inhibitory concentration being 250 µg/mL (Bandara *et al.*, 1989e).
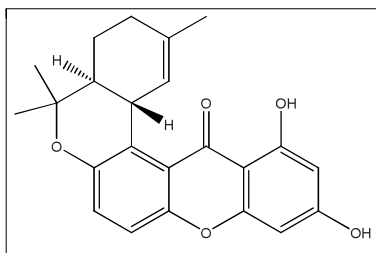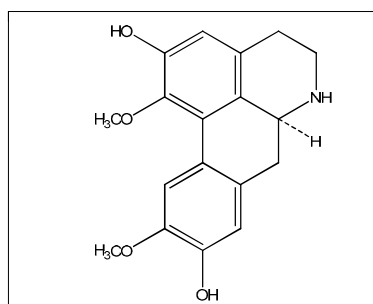

Figure 22: Structure of calozeyloxanthone
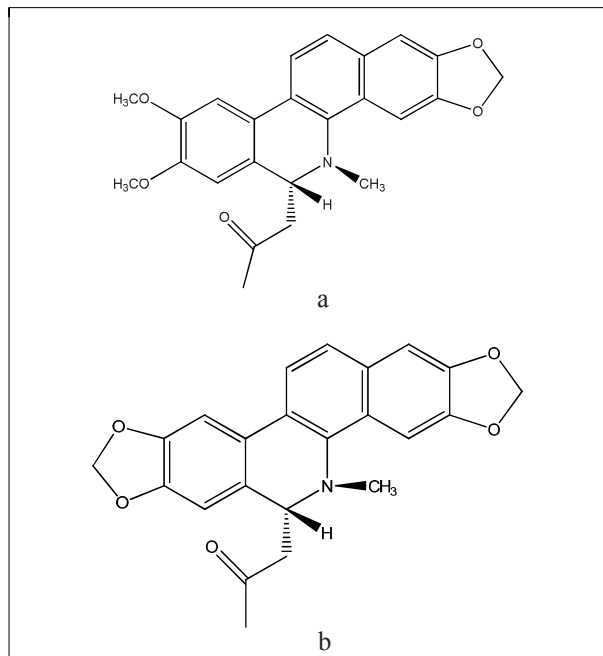

Figure 23: Structure of laurolitsine

Figure 24: Structure of (a) 8-acetonyldihydronitidine and (b) 8-acetonyldihydroavicine

Two benzophenanthrene alkaloids, 8-acetonyldihy-dronitidine (Figure 24a) and 8-acetonyldihydroavicine (Figure 24 b) have been isolated from *Zanthoxylum tetraspermum* Wight., stem bark along with liriodenine, sesamin, lichexanthone and (+)-piperitol-γ,γ-dimethylallylether. The *Zanthoxylum* species endemic to Sri Lanka, *Z. caudatum*, contains sesamin, savinin, liriodenine, decarine and 8-O-desmethyl-N-nornitidine. 8-Acetonyldihydronitidine and 8-acetonyldihydroavicine has shown significant growth inhibition of *S. aureus* (MIC value = 1.56 and 3.12 µg/mL, respectively). Furthermore, 8-acetonyldihydronitidine has exhibited strong antifungal activity against *C. cladosporioides* (area of inhibition 100 mm^2 at 2 mg) (Nissanka *et al.*, 2001).

Antifungal activity

Synthetic fungicides are currently used as the primary means for the control of plant disease.

However, alternative control methods are needed due to the negative public perceptions regarding the use of synthetic chemicals such as resistance to fungicides among fungal pathogens, and the high development cost of new chemicals. The use of plant-derived products as disease control agents have been studied, since they tend to have a low mammalian toxicity, less environmental effects and wide public acceptance (Lee *et al.*, 2007). Preventing fungal decay in organic fruits after harvest is an increasing challenge, and novel preservation approaches, which comply with organic food standards need to be developed.
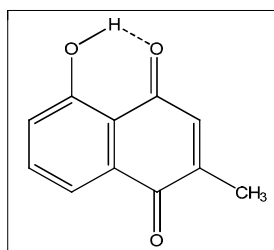

Figure 25: Structure of plumbagin

Plumbagin (Figure 25) is one of the bioactive metabolites found in *Plumbago zeylanica* L., *P. indica* and *P. europaea*. The antifungal activity of plumbagin against *Cladosporium cladosporioides*, *Alternaria tunis*, *Botrydiplodia theobromae*, *Aspergillus* sp., *Fusarium* sp., *Cercospora nicotinae*, and two isolates of *Colletotrichum gloeosporioides* obtained from avocado
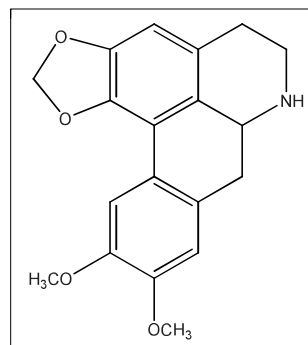
Figure 26: Structure of nordicentrine

and papaya has been determined using thin layer chromatography (TLC) bioassay. Plumbagin has shown higher antifungal activities against *A. tunis* (inhibition area = 4.5 cm^2), *B. theobromae* (inhibition area = 0.8 cm^2), *Fusarium* sp. (Inhibition area = 2.5 cm^2), and *C. gloeosporioides* (Inhibition area = 4.5 cm^2) compared to that of standard antifungal agent benlate (Adikaram *et al.*, 2002). On a spore germination assay conducted against *C. gloeosporioides*, a plumbagin solution at and above 31 µg/mL has caused total inhibition with an IC$_{50}$ value of 6.86 µg/mL, whereas at 50 µg/mL of the standard benlate required for the total inhibition (IC$_{50}$ = 44 µg/mL) (Adikaram *et al.*, 2002) was higher.
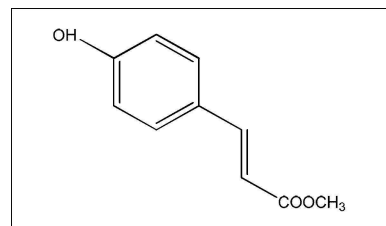
Figure 27: Structure of methyl-3-(4-hydroxyphenyl)-2E-propeonate
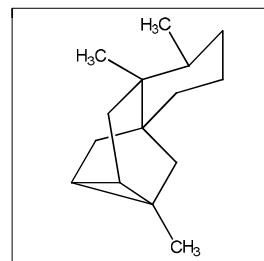
Figure 28: Structure of ishwarane

Discretine (Figure 1a) and nordicentrine (Figure 26), two alkaloids isolated from the stem bark and stem of *Xylopia championii* Hook., have been subjected to an antifungal

assay against *Clodosporium clodosporioides*. It was found that discretine was moderately active (MID = 30 µg/spot), while nordicentrine was highly active (6 µg/spot) when compared with the standard antifungal agent, benomyl, with an MID value of 1 µg/spot (Puvanendran *et al.*, 2008).

The methanol extract from *Costus speciosus* J. Konig., has led to the isolation of methyl-3-(4-hydroxyphenyl)-2E-propeonate (Figure 27) and the compound has inhibited the growth of *Aspergillus niger*, *C. cladosporioides, C. gloeosporioides*, *Curvularia* sp., and *Penicillum* sp. (Bandara *et al.*, 1988b).

From the hexane extract of the lichen *Heterrodermia microphylla*, atranorin (Figure 14) has been isolated and the results of quantitative spore germination assay indicated the antifungal activity of atranorin against *C. gloeosporioides* and *C. musae* with percentage germination values of 58.2 and 26.2, respectively, while the percentage germination for benlate was 33.3 (Bombuwala *et al.*, 2008).

Ishwarane (Figure 28) has been isolated from the CH$_2$Cl$_2$ extract of three *Hortonia* spp. *viz.*, *H. angustifolia*, *H. floribunda* and *H. ovalifolia*. This compound has displayed a moderate antifungal activity against *C. cladosporioides* with a 23 mm inhibition zone diameter compared with that of the standard antifungal agent benor (37 mm inhibition zone diameter) in TLC

bioassay. This activity has been further corroborated by using germination inhibition assay and found that Ishwarane totally inhibited the germination of *C. cladosporioides* spores at concentrations higher than 6.25 ppm (Ratnayake *et al.*, 2008).

In the search for active principles from the roots of *Eupatorium riparium* Regel, methylripariochromene A (Figure 29) has been isolated from *n*-hexane extract and was tested against *C. gloeosporioides*. It has exhibited antifungal activity comparable to that of the commercial fungicide, benlate in TLC bioassay. Results obtained from the germination inhibition bioassay corroborated that methylripariochromene A and benlate had the same degree of antifungal activity against *C. gloeosporioides* (Bandara *et al.*, 1992).

Methyl orsellinate (Figure 30) isolated from *Cladonia* sp., *H. obscurata* and *P. grayana* has shown significant antifungal activity against *T. longifusus*, *A. flavus*, *M. canis* and *F. solani*. Further, 80 % inhibition was observed for *T. longifusus*, *A. flavus* and *M. canis* while 70 % inhibition was observed for *F. solani* at a concentration of 200 µg/mL (Thadani *et al.*, 2012).

Methyl-β-orcinolcarboxylate (Figure 31) isolated from *Cladonia* sp. and *H. obscurata* showed the widest range of antifungal activity, being over 80 % active against *T. longifusus*, *A. flavus*, *M. canis*, *F. solani*, *C. glabrata* and *C. albicans* (Thadani *et al.*, 2012).
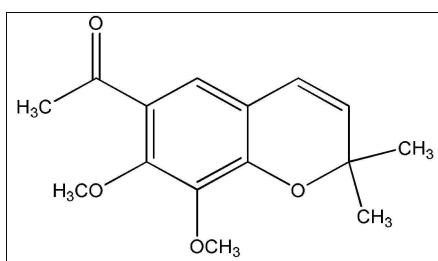

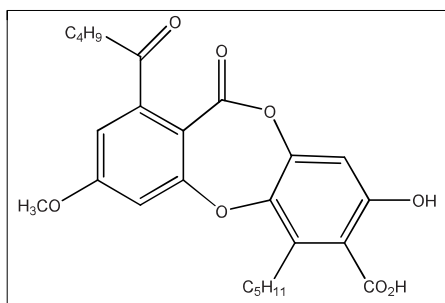**Figure 29:** Structure of methylripariochromene A
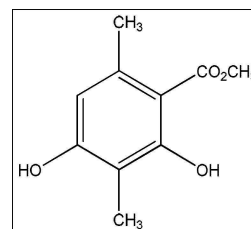

Figure 31: Structure of methyl-β-orcinolcarboxylate

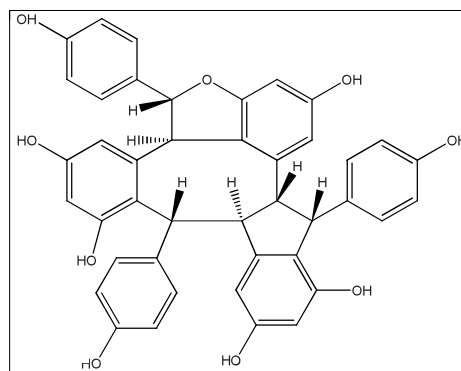**Figure 30:** Structure of methyl orsellinate

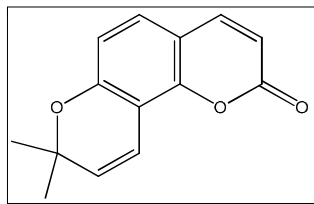**Figure 32:** Structure of canaliculatol

Figure 33: Structure of seselin

The acetone extract of the bark of *Stemonoporus canaliculatus* Thw. collected from the Kanneliya Forest in the south of Sri Lanka, has shown antifungal activity against the fungus *Cladosporium cladosporioides* in the TLC spray bioassay. The column chromatographic separation on silica gel has led to the isolation of canaliculatol (Figure 32), which has shown antifungal activity against the fungus *Cladosporium cladosporioides* (Bokel *et al.*, 1988).

The root bark of *Pleiospermium alatum* Wight & Arn., has afforded five alkaloids, five coumarins, lupeol and stigmasterol. One of the acridones, 1,5,6-trihydroxy-2,3-dimethoxy-10-methyl-9-acridone is a new record of a compound while another, 1-hydroxy-2,3,5,6-tetramethoxy-10-methyl-9-acridone, is a new record of a natural product. However, seselin (Figure 33), a coumarin, has displayed a significant antifungal activity against *Cladosporium cladosporioides* in TLC bioassay (Bandara *et al.*, 1990a).
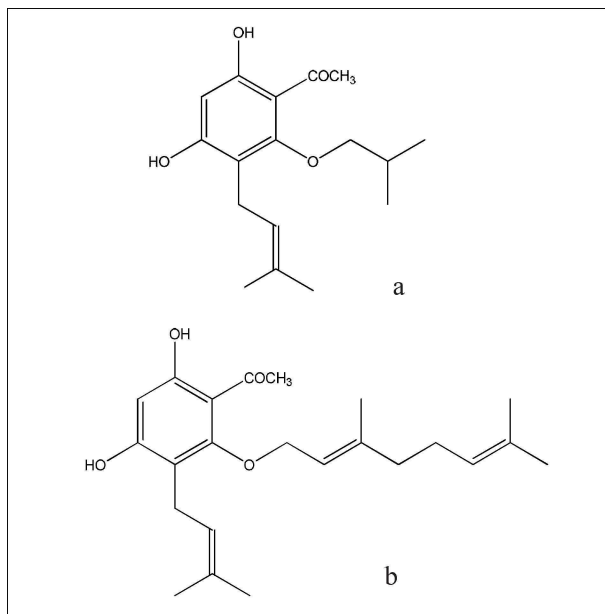
Figure 34: Structure of (a) 1-[2′,4′-dihydroxy-6′-(3″-methyl-2″-butenyloxy)-5′-(3″-methyl-2″-butenyl)]phenylethanone and (b) 1-[2′,4′-dihydroxy-6′-(3″,7″-dimethylocta-2″,6″-dienyloxy)-5′-(3″-methyl-2″-butenyl)] phenylethanone

Melicope lunu-ankenda (Gaertner) T. Hartley [*Euodia lunu-ankenda* (Gaertner) Merr.], a species found in Southern Asia, has been used in traditional medicine in Sri Lanka. The neutral fraction of the dichloromethane extract of *M. lunu-ankenda* has shown to be strongly active against the fungus *Cladosporium cladosporioides* in the TLC bioassay. Chromatography of the fraction has yielded two fungicidal phenylethanones, 1-[2′,4′-dihydroxy-6′-(3″-methyl-2″-butenyloxy)-5′-(3″-methyl-2″-butenyl)]phenylethanone (Figure 34a) and 1-[2′,4′-dihydroxy-6′-(3″,7″-dimethylocta-2″,6″-dienyloxy)-5′-(3″-methyl-2″-butenyl)] phenylethanone (Figure 34b) (Kumar *et al.*, 1990).
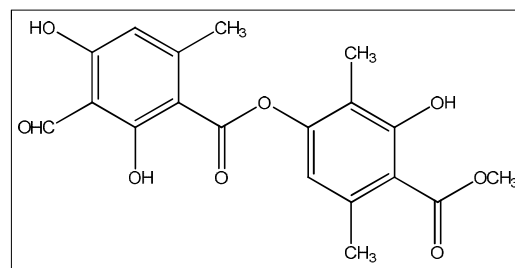
Figure 35: Structure of 3-formyl-2,4-dihydroxy-6-methylbenzoic acid 3-hydroxy-4-Z-methoxycarbonyl-2,5-dimethylphenyl ester

The hexane extract of the stem bark of *Gordonia dassanayakei* Wadhwa & Weeras., has shown a high antifungal activity against the plant pathogenic fungi *Curvularia* sp., *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Corynespora cassiicola*, and *Fusarium* sp.. A compound responsible for the antifungal activity has been isolated and identified as 3-formyl-2,4-dihydroxy-6-methylbenzoic acid 3-hydroxy-4-Z-methoxycarbonyl-2,5-dimethylphenyl ester (Figure 35). This active compound inhibited the growth of *Curvularia* sp., by 17.4 % at a concentration of 0.01 % while the standard compound, benelate inhibited the growth by 42.6 % at the same concentration. The percentage inhibition of growth by the active compound against *Rhizoctonia solani*, *Curvularia* sp., *Fusarium* sp., and *Colletotrichum gloeosporioides* were 9.3, 32.3, 14.3 and 20.7 %, respectively, whilst that by the standard antifungal agent benelate, were 28.4, 63.7, 53.7 and 34.8 %, respectively (Athukoralage *et al.*, 2001).
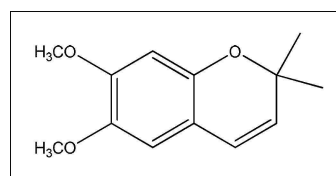
Figure 36: Structure of precocene II

Ageratum conyzoides L., is an annual herb that grows in the tropics and subtropics of which, the extracts are known to possess pharmacological and biocidal activity. The organic solvent extracts from the shoots have been tested for antifungal activity against the plant pathogenic fungi *Rhizoctonia solani, Sclerotium rolfsii, Botryodiplodia theobromae, Phomopsis theae and Fusarium* species growing *in vitro* on potato dextrose agar medium. The crude *n*-hexane extract completely inhibited the growth of *R. solani* and *S. rolfsii*. Activity guided fractionation of the *n*-hexane extract has yielded an antifungal compound precocene II (Figure 36), which exhibited 100 % inhibition of *R. solani* and *S. rolfsii* at a concentration of 80 – 100 ppm. The sclerotia of *R. solani* and *S. rolfsii* were also completely suppressed by precocene II at 150 ppm (Iqbal *et al.*, 2004).

The antifungal activity guided fractionation of the *n*-butanol extract of the methanol extract of the stem bark of *Artocarpus nobilis* Thwaites has furnished two stilbene derivatives (E)-4-isopentenyl-3,5,20,40-tetrahydroxystilbene (Figure 3a) and (E)-4-(3-methyl-E-but-1-enyl)-3,5,20,40-tetrahydroxystilbene (Figure 3b). Both compounds have shown strong antifungal activity at 10 μg/spot against *Cladosporium cladosporioides* on TLC bio-autography method.

Antifungal activity guided fractionation of solvent extracts of the stem bark of *Bridelia retusa* Spreng., belonging to the family Euphorbiaceae against *Cladosporium cladosporioides* furnished new bisabolane sesquiterpenes (E)-4-(1,5-dimethyl-3-oxo-1-hexenyl)benzoic acid (Figure 37a), (E)-4-(1,5-dimethyl-
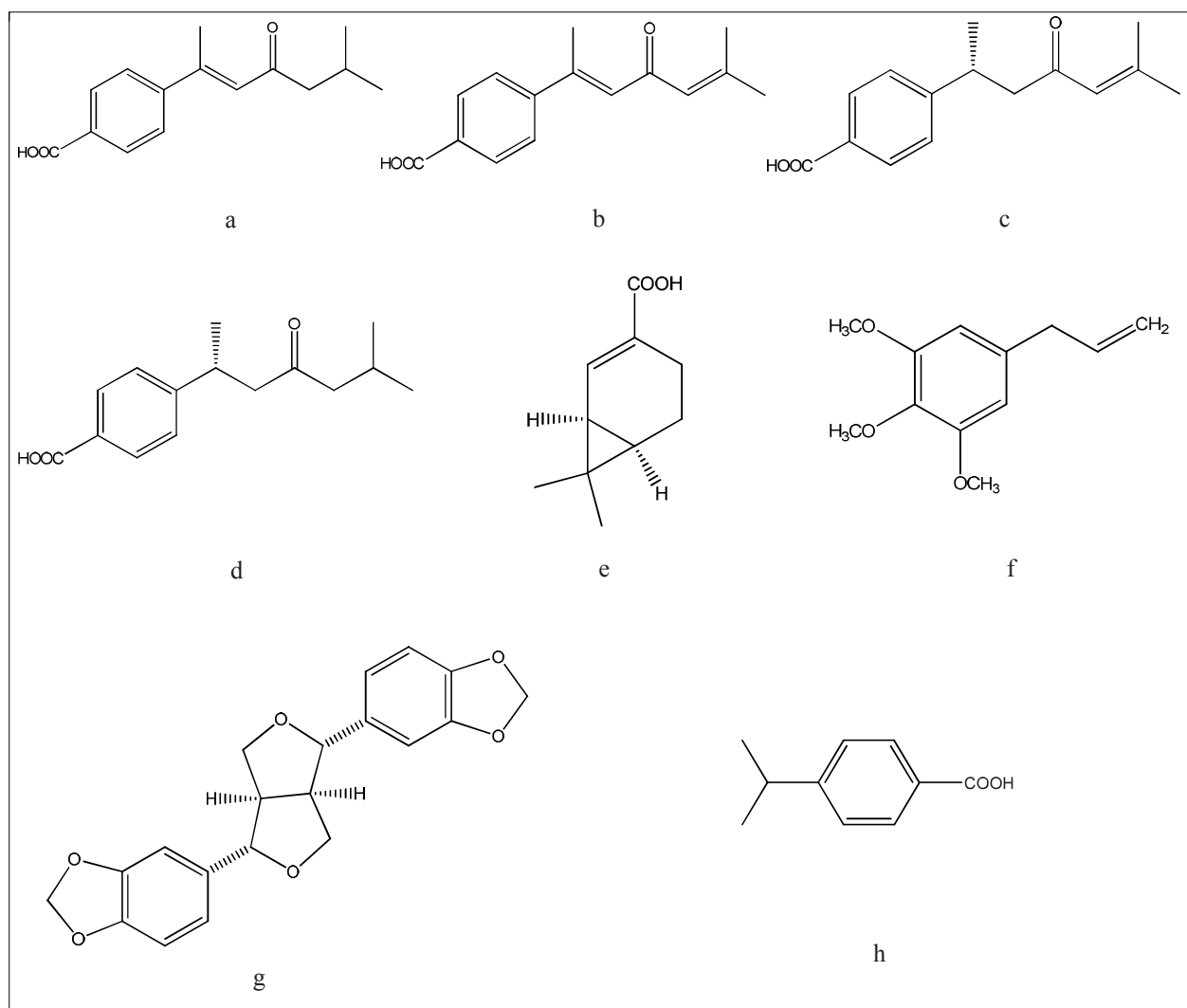
Figure 37: Structure of (a) (E)-4-(1,5-dimethyl-3-oxo-1-hexenyl)benzoic acid; (b) (E)-4-(1,5-dimethyl-3-oxo-1,4-hexadienyl) benzoic acid; (c) (R)-4-(1,5-dimethyl-3-oxo-4-hexenyl)benzoic acid; (d) (-)-isochaminic acid; (e) (R)-4-(1,5-dimethyl-3-oxohexyl)benzoic acid (ar-todomatuic acid);, (f) 5-allyl-1,2,3-trimethoxybenzene (elemicin); (g) (+)-sesamin and (h) 4-isopropylbenzoic acid

3-oxo-1,4-hexadienyl) benzoic acid (Figure 37b), (R)-4-(1,5-dimethyl-3-oxo-4-hexenyl)benzoic acid (Figure 37c) and (–)-isochaminic acid (Figure 37d), together with the known (R)-4-(1,5-dimethyl-3-oxohexyl)benzoic acid (ar-todomatuic acid) (Figure 37e), 5-allyl-1,2,3-trimethoxybenzene (elemicin) (Figure 37f), (+)-sesamin (Figure 37g) and 4-isopropylbenzoic acid (cumic acid) (Figure 37h). All these compounds have shown fungicidal activity on TLC bioautography method at very low concentrations except elemicin (Jayasinghe *et al.*, 2003).
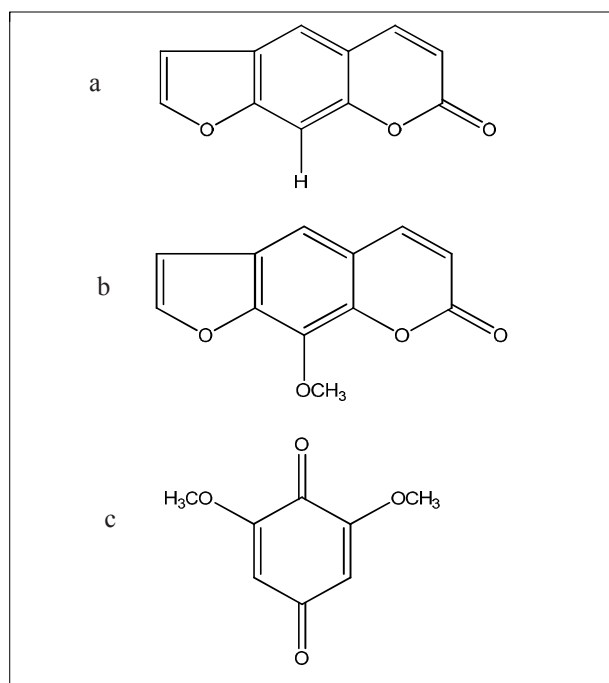

Figure 38: Structure of (a) psoralene; (b) xanthotoxin and (c) 2,6-dimethoxybenzoquinone

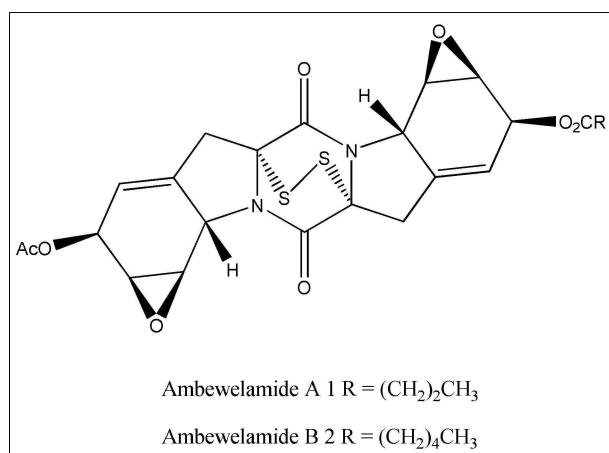Ambewelamide A 1 R = (CH₂)₂CH₃

Ambewelamide B 2 R = (CH₂)₄CH₃

Figure 39: Structure of ambewelamide A and B

The unripened fruit of *Limonia acidissima* L., was extracted into chloroform and the TLC *Cladosporium* bioassay showed three compounds, psoralene (Figure 38a), xanthotoxin (Figure 38b) and 2,6-dimethoxybenzoquinone (Figure 38c), which were able to inhibit the growth of *Cladosporium* (Adikaram *et al.*, 1989).

Anticancer activity

Cancer has no geographic, national, religious or other boundaries. Each year over 6.5 million people are diagnosed with cancer worldwide (Culotta & Koshland, 1993). Natural products and their semisynthetic analogues have provided some of the most effective and widely used chemotherapeutic agents for cancer (Cordel, 1978). This is exemplified by such compounds as Taxol ®, the *Vinca* alkaloids, vinblastine, vincristine, the podophyllotoxin analogues etoposide and teniposide, the camptothecin analogues topotecan, and CPT-11, the microbial products bleomycin, mitomycin C, the anthracyclines adrimycin and daunorubicin. Importantly, these anticancer compounds are highly complex that they would not have emerged from a synthetic programme alone or from the combinatorial approach to drug discovery. Thus, natural products approach continues to be important for the development of new anticancer drugs as it is complementary to synthetic and biosynthetic approaches.

Two new members of a family of highly modified phenylalanine diketopiperazine, ambewelamide A and B (Figure 39) have been isolated from the CH₂Cl₂ extract of *Usnea* sp., found in Ambewela, Sri Lanka. Of the two compounds, ambewelamide A exhibited potent *in vitro* cytotoxicity against murine leukemia P388 cells with an IC_{50} value of 8.6 ng/mL and showed significant *in vitro* antineoplastic activity against P388 cells (percentage T/C 140 at 160 µg/kg) (Williams *et al.*, 1998).

A flabelliferin (Figure 40) isolated from the methanol: water (1:1) extract of *Borassus flabellifera* L., flour was introduced into micro wells containing melanoma cells. A 13 % inhibition of the cell growth has been observed for the isolated compound at the concentration of 100 µg/mL on the melanoma cell line, while the reference compound, doxorubicin resulted in a 63 % inhibition of cell growth at the concentration of 20 µg/mL (Keerthi *et al.*, 2009).

Seselin (Figure 33) isolated from *Pleiospermium alatum* Radlk., has shown detectable activity against a yeast strain rad 52, at a dose of 500 µg/mL. Further, seselin has shown selective activity against the rad 52 yeast strain as compared with the wild-type RAD⁺
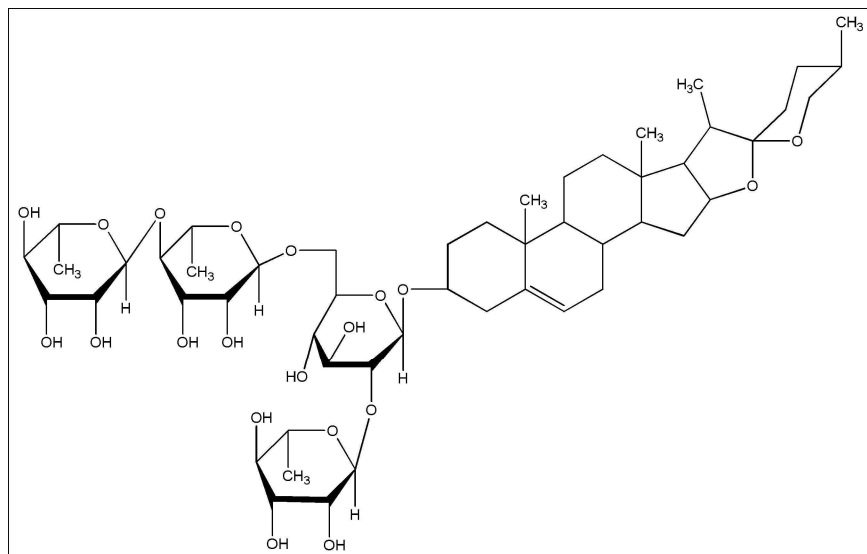
Figure 40: Structure of flabelliferin
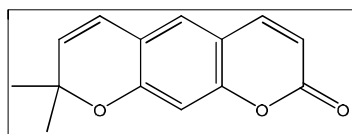
Figure 41: Structure of xanthyletin
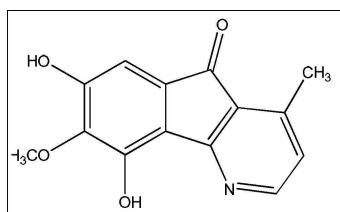
Figure 42: Structure of cyathocaline

strain, indicating that it functions as a DNA-damaging agent. Seselin was also weakly active in a mammalian cytotoxicity assay against Vero monkey cells, with an IC_{50} value of 12 µg/mL (Gunatilaka *et al.*, 1994).

The other compound isolated from *Pleiospermium alatum* was xanthyletin (Figure 41), which has shown somewhat higher activity in the wild-type RAD^+ cell line (IC_{50} value = 21 µg/mL) suggesting that its cytotoxicity is due to some other mechanism than the DNA damage (Gunatilaka *et al.*, 1994).

Cyathocalyx zeylanica Champ. ex Hook., is a plant belonging to the family Annonaceae. Bioactivity-guided fractionation of the methanol extract of the

stem bark of *C. zeylanica* has afforded a moderately bioactive new azafluorenone alkaloid, cyathocaline (Figure 42), the structure of which was established as 5,7-dihydroxy-6-rnethoxy-l-methyl-4-azatluoren-9-one. Cyathocaline exhibited moderate but selective activity in the mechanism-based yeast bioassay for DNA-modifying agents with the following IC_{50} values: RS 322 YK (*rad*52Y), 90 µg/mL; RS 321 N, 87 µg/mL; RS 188 N (RAD^+) > 400 µg/mL; inactive in the RS 167 N(rad6) strain. In a cytotoxicity test against the A-549 human lung carcinoma cell line, it had an IC_{50} value of 8.5 ± 0.07 µM (Wijeratne *et al.*, 1995).

The stem bark of *Pleurostylia opposita* Wall., has been extracted with $MeOH/CH_2Cl_2$ (1:1 v/v). Size extrusion of the purified extract yielded two sesquiterpene pyridine alkaloids, oppositines A (Figure 43a) and B (Figure 43b). Oppositines A and B when exposed to the cancer cell line HCT-116 (human colon tumor) have shown moderate cytotoxicity against HCT116 cell lines with EC_{50} values of 27 ± 2 and 26 ± 3 µM, respectively (Whitson *et al.*, 2006).

The lichen *Parmotrema* sp. (Parmeliaceae), collected from Labukelle, Central Province, Sri Lanka, has been sequentially extracted into methanol and the extract has shown significant *in vitro* kinase inhibitory activity. Bioassay-guided fractionation of the *Parmotrema* sp., extract has led to the isolation of depside inhibitors. A new depside (Figure 44a) has been isolated along with two known metabolites, β-collatolic acid (Figure 44b) and β-alectoronic acid (Figure 44c). These three depsides, depside β-collatolic acid and β-alectoronic acid have

Figure 43: Structure of (a) oppositines A and (b) oppositines B

Figure 44: Structure of (a) new depside; (b) β-collatolic acid and (c) β-alectoronic acid

Figure 45: Structure of (a) salacinol and (b) kotalanol

exhibited moderate inhibition of purified recombinant Plk1 kinase with IC_{50} values of 2.8, 0.7, and 1.7 μM, respectively at 1 μM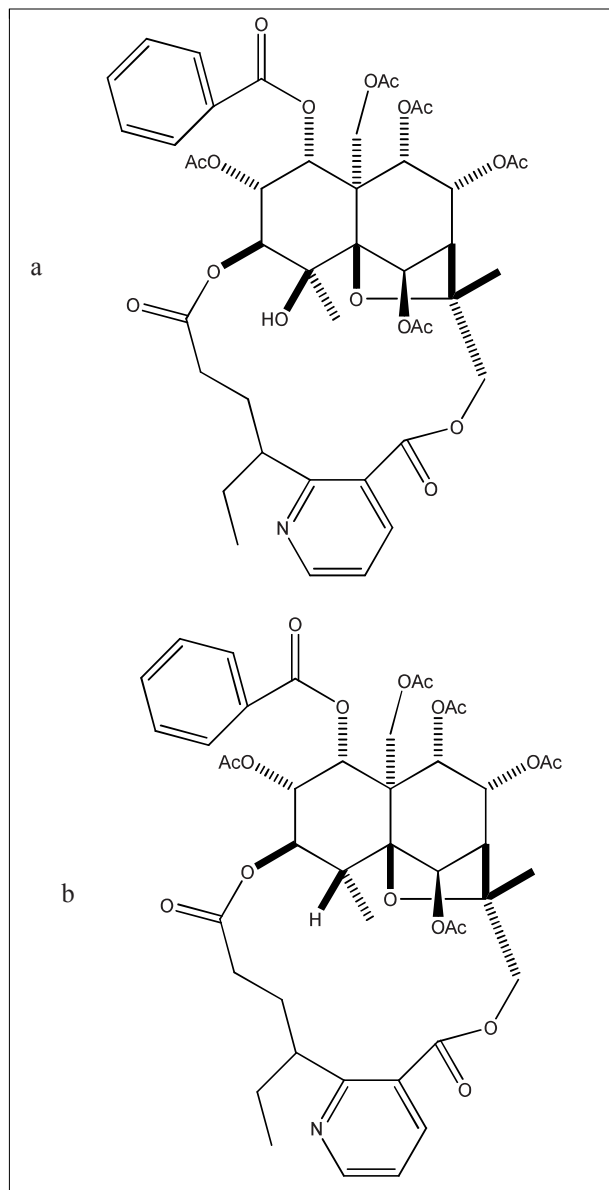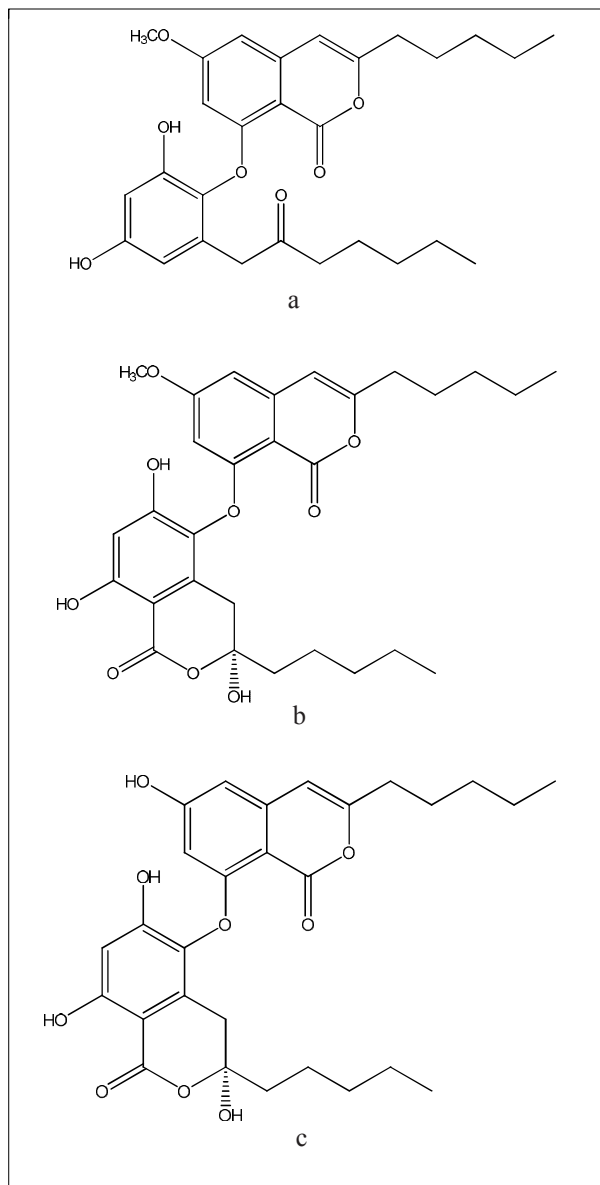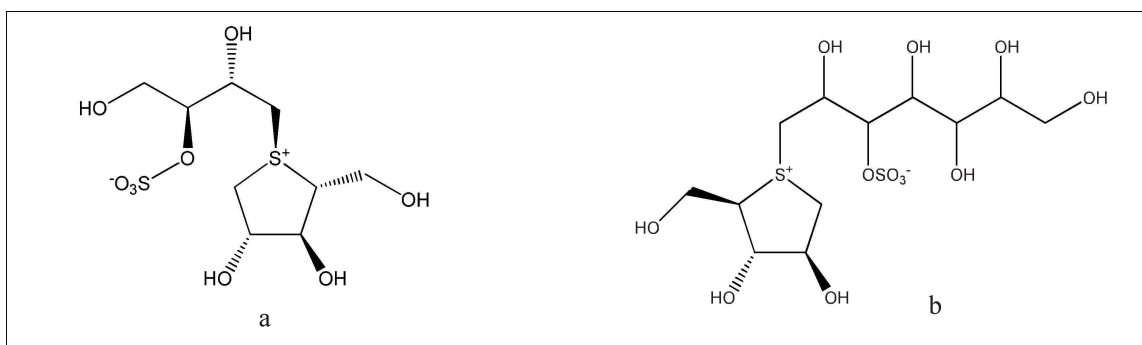 ATP. The inhibitory activity has been also observed at high ATP concentrations, suggesting the potential for activity in a cellular environment. The depsides when tested against a panel of 23 other recombinant inases were found to possess up to 30-fold selectivity toward Polo-like kinase-1 (Williams *et al.*, 2011).

Oral hypoglycaemic activity

Diabetes mellitus is a chronic disorder of carbohydrate, fat, and protein metabolism (Anirban & Abdul, 2005). A defective or deficient insulin secretory response causes impaired carbohydrate (glucose) use and the resultant hyperglycaemia is a typical feature of diabetes mellitus. It is the fourth leading cause of death in developed countries and diabetes mellitus affects more than 5 % of the world's population, making it one of the most common non communicable diseases (2001). There are more than 220 million persons affected with diabetes in the world today, and by 2025, the number is expected to approach 300 million (Simon, 2010). A standard treatment uses drugs such as sulphonyl ureas, biguanides and thiazolidinediones, which reduce hyperglycaemia by inducing β-cells to produce more insulin. However, undesired consequences of prolonged use of sulphonylureas and other drugs include hypoglycaemic episodes, ultimate exertion of β-cell and long term angiogenic side effects, which result due to chronic exposure to increased insulin levels (Bebernitz *et al.*, 2001).

A large number of medicinal plants used in the control of diabetes mellitus have been reported (Bailey & Day, 1989; Marles & Farnsworth, 1994). Although these plants may represent alternatives to developing new oral hypoglycaemic agents, appropriate and unambiguous ethnobotanical information is needed to exploit them.

Salacia reticulata Wight., is an indigenous plant, which grows in the Dry Zone forests of Sri Lanka. Known as kothala himbutu, this herb is used for the treatment of diabetes in ayurvedic medicine. A polyhydroxylated cyclic 13-membered sulfoxide, salacinol (Figure 45a) has been isolated from an aqueous extract of *S. reticulata* through bioassay guided separation. Salacinol has shown potent inhibitory activities on several α-glucosidases such as maltase, sucrase and isomaltase, and the inhibitory effects on serum glucose levels in maltose and sucrose-loaded rats (*in vivo*) have been found to be more potent than that of acarbose, a commercial a-glucosidase inhibitor. The α-glucosidase

inhibitory activity of salacinol is also reported (IC_{50}: maltase, 3.2 mg/mL; sucrase, 0.84 mg/ mL; isomaltase, 0.59 mg/mL). Interestingly, the organic extracts of the plant contained triterpenoids (Gunatilaka *et al.*, 1993; Tezuka *et al.,* 1993; 1994; Dhanabalasingham *et al.*, 1996).

A potent natural α-glucosidase inhibitor, kotalanol (Figure 45b) (IC_{50}: maltase, 2.8 mg/mL; sucrase, 0.58 mg/mL; isomaltase, 1.9 mg/mL) has been isolated from the roots and stems of *S. reticulata*.

Several studies on the chemistry of *S. reticulata* have been conducted using *S. reticulata* var. *diandra*, a variety endemic to Sri Lanka (Karunaratne, 2013). According to the current literature (Dassanayake, 1996), this variety is now elevated to the species level (*S. diandra*).

α -Glucosidase inhibitory activity

α-Glucosidase inhibitors have proved useful in the reduction of postprandial hyperglycaemia by suppressing the absorption of glucose, being effective in the treatment of type II diabetes and obesity (Raskin *et al.*, 2007). The current interest in these compounds has been extended to a diverse range of diseases, including lysosomal storage disorders, cancer and special attention has been given to those compounds with anti-HIV activity (Kouam *et al.*, 2006). Because of their promising therapeutic potential, *α*-glycosidase inhibitors are being searched from natural
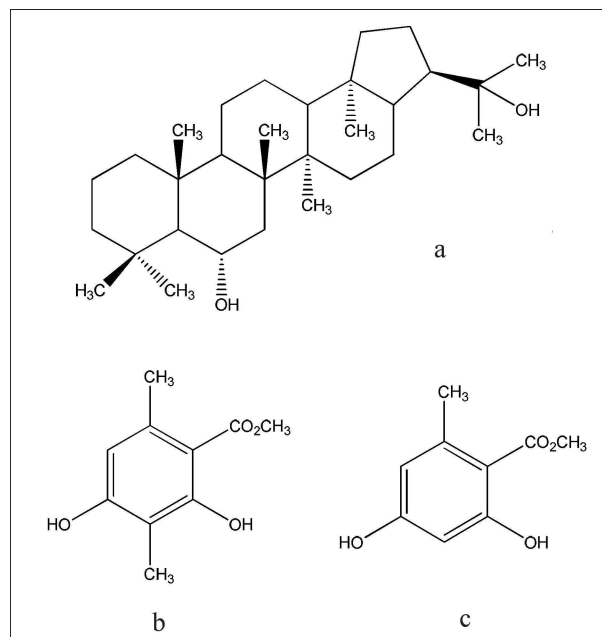

Figure 46: (a) zeorin; (b) methyl-β-orcinol carboxylate and (c) methyl orsellinate

sources. Polyhydroxy glycosidase inhibitors are a widely diverse class of compounds often isolated from plants and microorganisms, which have significant therapeutic potential (Melo *et al.*, 2006). Carbocyclic compounds include potent HIV inhibitors such as conduritol epoxides and aminoconduritols, while conduritol A analogues modulate the release of insulin (Abbasi *et al.*, 2005). Thiosugars, either synthesized or isolated from natural sources, have also been investigated as inhibitors and have widened the structural diversity of such compounds available from natural sources. Compounds with no obvious structural similarity to a carbohydrate skeleton are a new class of inhibitors and the elucidation of their mechanism of action may add new insights in the search for new therapeutic agents (Melo *et al.*, 2006).

Three compounds, zeorin (Figure 46a), methyl-β-orcinol carboxylate (Figure 46b), and methyl orsellinate (Figure 46c), have been isolated from dichloroform and methanol fractions of dried lichens *Parmolrema grayana* and *Cladonia* sp.. These three compounds, zeorin (IC_{50} = 100.0 ± 0.3 µM), methyl-β-orcinol carboxylate (IC_{50} = 140.0 ± 0.6 µM), and methyl orsellinate (IC_{50} = 165.0 ± 1.2 µM), has shown tremendous α-glucosidase inhibitory activity showing lower IC_{50} values compared to the standard compounds 1-deoxynojirimycin (IC_{50} = 425.0 ± 8.9 µM) and acarbose (IC_{50} = 700.0 ± 10.4 µM) (Thadani *et al.*, 2011).

Sperm motility enhancing activity

Globally, infertility affects about 50 to 80 million couples at some point of their reproductive lives with a variety of biological and behavioural determinants (World Health Organisation, 2003).There is a need and a demand for new sperm stimulants to be used in asthenozoospermia and in some assisted reproductive programmes. In many parts of the world, efforts are now being aimed at investigating the therapeutic efficacy of locally available medicinal herbal plants. The beneficial role of medicinal plants in the treatment of male infertility has been numerously indicated (Saalu *et al.*, 2006; 2009a; 2009b; 2010).

The development of new fertility regulating drugs from medicinal plants is an attractive proposition. Natural plant substances possessing mild inherent estrogenic or anti-estrogenic properties offer themselves as effective non-conventional sources of contraception with less deleterious side effects. Many plants and herbs have also been reported to have potential antifertility properties (Casey, 1960). Many of these plant products having inherent estrogenic or anti-estrogenic effects possibly bring about alterations in tubal transport of

blastocyst or hormonal milieu of the uterus making the uterine environment hostile for implantation or fetal development.

A steroid glycoside 1 (Figure 47) isolated from Sri Lankan soft coral *Sinularia crispa* showed a spermatostatic activity on rat cauda epididymal spermatozoa (Tillekeratne *et al.*, 1989).

A non-steroidal contragestative agent (Figure 48) has been isolated from the MeOH-CH_2Cl_2 (1:1) crude extract of the Sri Lankan marine red algae, *Gelidiella acerosa*. Contragestative effects of this compound assessed in pregnant rats has shown 80 % contragestative activity without any overt clinical signs of toxicity (Premakumara *et al.,* 1996).
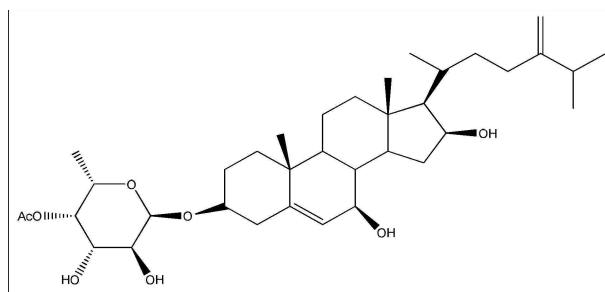

Figure 47: Structure of steroid glycoside 1 from Sri Lankan soft coral *Sinulariacrispa*
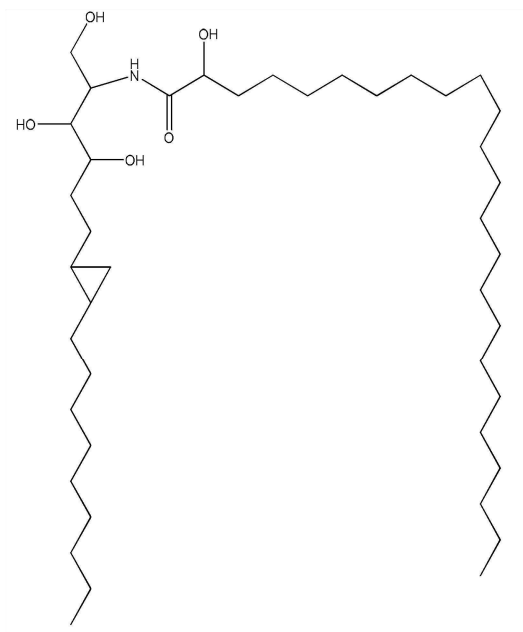

Figure 48: Structure of non-steroidal contragestative agent isolated from Sri Lankan soft coral *Sinulariacrispa*

Anti-inflammatory activity

Although, no bioactive compounds in this category have been isolated so far, Sri Lankan studies on the anti-inflammatory activity is significant because bioassays carried out on crude extracts have shown the potential of Sri Lankan plants having anti-inflammatory activity.

Leaves of *Argyreia populifolia* Choisy (Convolvulaceae) is used in the traditional medicine for inflammation in joints and arthropod bites. The anti-inflammatory activity of the leaves have been evaluated using carrageenan-induced paw oedema test in rats. A 10 mL/kg oral dose of fresh juice of the leaves significantly impaired the initial phase of the acute inflammatory response (by 33%) in this model. The juice has shown a marked anti-histamine activity when assessed using histamine-induced vascular permeability test. It has been concluded that the leaf juice has an anti-inflammatory activity mediated *via* antihistamine action, justifying its use in traditional medicine (Ratnasooriya & Dharmasiri, 2001).

The anti-inflammatory potential of an aqueous leaf extract (ALE) of *Ixora coccinea* (Rubiaceae) in rats after oral administration (500, 1000 and 1500 mg/kg) has also been investigated. This has been done using the carrageenan-induced paw oedema (acute inflammatory model) and cotton pellet granuloma tests (chronic inflammatory model). In the former test, ALE significantly reduced both early and late phases of the inflammatory response and also the oedema maintained between the two phases. In the latter test, it has significantly suppressed granuloma formation (only highest dose tested). Collectively, the results has shown a promising anti-inflammatory activity against both acute and chronic inflammation (Ratnasooriya *et al.*, 2005). The anti-inflammatory activity of methanolic leaf extract (MLE) of *I. coccinea* Linn. (Rubiaceae), has also been investigated. It has been found that the methanolic leaf extract has a dose-dependent anti-inflammatory activity in carrageenan-induced rat paw oedema model ($r^2 = 0.7$; $p < 0.01$) (Handunnetti *et al.*, 2009).

Trichosanthes cucumerina Linn. (Cucurbitaceae), is one of the medicinal plants often used in Sri Lankan traditional medicine. The anti-inflammatory activity of the hot water extract and its fractions of *T. cucumerina* have been evaluated by the use of carrageenan-induced paw oedema model in Wistar rats. Apart from the lowest dose of the HWE, other tested doses (500, 750, 1000 mg/kg) produced a significant ($p \leq 0.05$) inhibition

of the inflammation, most pronounced at 5 hours after the injection of carrageenan. The anti-inflammatory effect induced by 750 mg/kg was comparable to that of the reference drug, indomethacin at 4 and 5 hours (Arawwawala *et al.*, 2010).

Vitex negundo L. (Verbenaceae) is a small tree, of which the water extract of fresh mature leaves is used internally and externally in ayurveda medicine as anti-inflammatory, analgesic and anti-itching agents. The early phase (2 hours) of carrageenan-induced rat paw oedema was significantly ($p < 0.01$) suppressed in an inversely does-dependent ($r^2 = 1$, $p < 0.01$) manner by fresh mature leaf extract ($EC_{50} = 2$ g/kg). In the formaldehyde-induced rat paw oedema test, the 2.5 and 5 g/kg leaves have significantly ($p < 0.05$) suppressed the inflammation on days 4 – 6 of the test (Dharmasiri *et al.*, 2003).

The anti-inflammatory potential of Sri Lankan black tea (*Camellia sinensis* L.) (Theaceae) has been determined using both acute (carrageenan-induced paw oedema) and chronic (formaldehyde-induced paw oedema and cotton pellet granuloma test) rat inflammatory models. Three doses of black tea brew (BTB) [84 mg/mL, equivalent to 1.5 cups; 168 mg/mL, equivalent to 3 cups; and 501 mg/mL, equivalent to 9 cups] made using high grown unblended dust grade No: 1 black tea samples were orally administered to rats (n = 6 – 9/ dose/test). The results have shown that Sri Lankan BTB possesses marked and significant ($p < 0.05$) oral anti-inflammatory activity against both acute and chronic inflammation. This anti-inflammatory activity was dose-dependent in the carrageenan-induced paw oedema test and cotton pellet granuloma test. Further, in the carrageenan paw oedema model, the anti-inflammatory activity of BTB was almost identical to the green tea brew of both Chinese and Japanese types (Ratnasooriya & Fernando, 2009).

In Sri Lankan traditional medicine a decoction of leaves and stems of *Anisomeles indica* (Lamiaceae) is claimed to possess anti-inflammatory activity. Three doses of the freeze-dried decoction of a pre-flowering plant (E1) (125, 250 and 500 mg/kg) and one dose of the decoction of a plant at flowering stage (E2) (500 mg/kg) were orally administered to rats. The anti-inflammatory activity has been evaluated using the carrageenan-induced paw edema, formaldehyde-induced paw oedema and adjuvant-induced paw oedema models in rats. E1 demonstrated a significant ($p < 0.01$) and dose-dependent anti-inflammatory effect in all three models, while E2 did not demonstrate significant anti-inflammatory activity (Dharmasiri *et al.*, 2002).
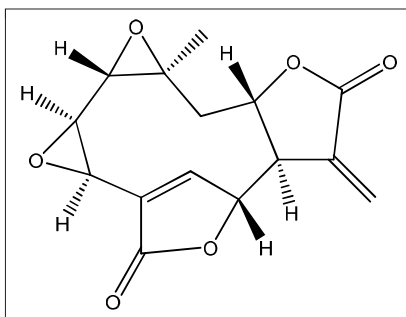
Figure 49: Structure of mikanolide

Miscellaneous activity

Mikania scandens B. L. Rob., is a common invasive plant in Sri Lanka and other South Asian countries. Preliminary investigation of a number of plant extracts for allelopathic activity using seed germination inhibition bioassay has shown promising bioactivity of the water extract of the aerial parts of *M. scandens*. Activity-guided fractionation of the *M. scandens* extract led to the isolation of the highly allelopathic active compound mikanolide (Figure 49), with an MIC of 0.083 µM/mL. This plant could be used to develop as an environment friendly natural herbicide, either in crude form as shredded plant material or as pure mikanolide, which is the major constituent (~ 0.02 %) in the plant (Piyasena & Dharmaratne, 2013).

CONCLUSION

Bioactive compounds ranging from alkaloids, terpenoids, aromatic compounds and lichen specific metabolites isolated from Sri Lankan flora exhibits the potential of the Sri Lankan flora as an economic resource. In light of these findings, a comprehensive investigation of bioactive compounds of Sri Lankan endemic plants has now become of prime importance.

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