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Anti-Respiratory Syncytial Virus Agents from Phytomedicine

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1. Introduction

Although the global importance of RSV as a respiratory pathogen has been recognized for over 40 years, suitable prophylactic and therapeutic interventions have not been truly available. Vaccine development, unfortunately, has been fraught with spectacular failure and with difficult obstacles, and there are only limited therapeutic options for treatment of this disease. Currently, the only approved prophylactic options available involve the use of Palivizumab and its derivative Motivizumab (currently under trial) which are both monoclonal antibodies directed against RSV surface fusion protein. Ribavirin, a broad-spectrum anti-viral agent, is the only therapeutic option employed as adjunctive therapy for the sickest patients; however, its efficacy has been called into question by multiple studies, and most institutions no longer use it. Moreover, the use of both agents has been shown to be costly and difficult to handle. Therefore, the search for novel anti-viral inhibitors of RSV has become more intensive. It could be recalled that potent anti-viral agents have previously been harnessed from medicinal plants. Since medicinal plants have consistently served as suitable lead sources for potent anti-viral agents, efforts have also been made by several investigators in developing anti-RSV compounds from phytomedicine. In this present research paper, we present a review of the past to present activities involving the discovery and development of novel and effective anti-RSV compounds from phytomedicine. First, we begin by briefly describing the problem of the global disease burden of RSV as well as efforts and approaches so far adopted to contain the viral menace. In the next section we introduce the subject of phytomedicine and anti-RSV therapeutic products originating from various reported medicinal plants. Straightforward and discreet description is made of investigations carried out by our workgroup in our attempt to develop anti-RSV compounds from medicinal plants especially *Ramalina farinaceae* and *Aglaia ignea*. Here also in this section, we present and discuss published results from other investigators regarding compounds from other medicinal plant sources. The importance of the process and techniques leading to their identification and isolation is highlighted. Attention is drawn to

some common markers that characterize their discovery. We devote tables to give comprehensive listings and profile of these agents and also discuss other relevant results. Attempt is made to shed adequate light on the therapeutic efficacy to safety profile of promising compounds since the overall relevance of the compounds and their derivatives should depend largely on their efficacy-safety characteristics. Additionally, space is devoted to discuss the proffered or validated mechanistic bases of the observed the anti-viral and disease inhibitory activities of the phytoconstituents and compounds. The importance of structural modifications is equally reflected where applicable to grant a quick and early preview on the more likely positive alteration direction that could possibly select for enhancement of efficacy and cellular compatibility. Lastly, we discuss in the next section the promises and the crucial position occupied by anti-RSV compounds and phyto-constituents harnessed form phytomedicines in the treatment and control of RSV infection and disease. In conclusion, we comment on the future of RSV infection and disease control; the role that should expectedly be occupied by chemotherapy, especially phytomedicines-derived. In answering the questions of discovering and developing the tomorrow's effective anti-RSV compounds, we make some concluding remarks on some key performance indicators that should characterize the phytocompounds of desirable anti-RSV activity.

1.1 Respiratory syncytial virus

Respiratory syncytial virus (RSV) which belongs to the *Pneumovirus* genus of the Paramyxoviridae family is the most important cause of viral lower respiratory tract illness (LRI) in infants and children worldwide (Collins *et al*. 1996; Hall, 1994). Amongst children in the US, up to 125,000 RSV-associated hospitalizations and 500 RSV-associated deaths respectively could occur each year (Langley and Anderson, 2011). RSV was, on average, responsible for 17% of acute respiratory infections in children admitted to hospital in the developing countries (Martins *et al. 1998*), and studies from Africa have equally reported the influence of malnutrition on the prevalence of RSV (Adegbola *et al.* 1994; Nwankwo *et al.* 1994). In Nigeria it is reported that RSV infections occur all year round with a peak during the rainy season (Nwankwo *et al*. 1994). Infants who are premature (Berkovich, 1964; Cunningham *et al*. 1991) or have chronic lung disease (Groothuis *et al*. 1988) or congenital heart disease (MacDonald *et al*. 1982) are at particular risk for severe RSV disease. Although traditionally regarded as a pediatric pathogen, RSV can also cause life-threatening pulmonary disease in bone marrow transplant recipients (Fouillard *et al*. 1992) and the elderly (Dowell *et al*. 1996; Falsey *et al*. 1992, 1995, 2005; Falsey and Walsh, 1998). Although the global prevalence of RSV infection especially among infants and young children is on the increase, vaccine development, unfortunately, has been fraught with spectacular failure and with difficult obstacles, and there are only limited therapeutic options for treatment of this disease (Collins *et al*., 1996; Wright *et al*., 2000; Kohlmann et al., 2009; Tregoning and Schwarze *et al*., 2010). Therefore, the search for novel anti-viral inhibitors of RSV has become more intensive.

1.2 Phytomedicine and anti-viral agents

Natural products from plants traditionally have provided the pharmaceutical industry with one of its most important sources of lead compounds and up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version. Currently, over a 100 new products are in clinical development, particularly as anti-cancer

agents and anti-infectives (Gautam *et al*., 2007; Harvey, 2008; Jassim and Naji, 2003). This has influenced many of pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. The bioactive molecules occur in plants as secondary metabolites and as defense mechanisms against predation, herbivores, fungal attack, microbial invasion and viral infection. During the past decade, potent agents have become available against viral infections. Therefore, extracts of plants and phytochemicals are getting more important as potential sources for viral inhibitors during the recent decade. Extensive studies have shown that medicinal plants of several parts of the world contain compounds active against viruses that cause human diseases (Kott *et al*., 1999; Semple *et al*., 1998; Sindambiwe *et al*., 1999). Correspondingly, several potent agents against the respiratory virus- RSV have been reported. The aim of this review is to give a comprehensive outlook on the available and emerging promising phyto-constituents effective against RSV and an overview of the reported associated researches done so far. This review encompassed introduction, methodology, outcomes, and overall promises of the discovered putative anti-RSV agents. Furthermore, useful guiding components for future discovery and analysis of promising anti-RSV candidates are equally highlighted.

2. Plant species possessing anti-respiratory syncytial virus anti-viral activities

2.1 Aglaia species

Aglaia represents the largest genus within the family Meliaceae and contains more than 100 species (Bohnenstengel *et al*. 1999; Pannell 1992). It is a woody small or medium- sized tree found mostly in Southeast Asia. Extracts or pure compounds from the various species have been shown to display diverse biological activities ranging from anti-proliferation, anti-inflammatory, fungicidal, bactericidal, anthelminthic and anti-viral activity (Bohnenstengel *et al*. 1999; Lipipun *et al*. 2003; Perry 1980; Poehland *et al*. 1987). Although *Aglaia species* are traditionally used in Southeast Asia and Indo-China for the treatment of various diseases, including ailments related to lower respiratory tract infection and inflammation (Lipipun *et al*. 2003; Perry 1980), anti-viral screening with Aglaia species has essentially been limited to Herpes Simplex Virus types 1 and 2 (Lipipun *et al*. 2003; Poehland *et al*. 1987). Given that RSV infection has a strong bearing with inflammation of the lower respiratory tract, we decided to explore various compounds from these species for possible anti-RSV activities *in vitro*.

Our investigation (Esimone *et al*., 2008) involving the isolation and screening of eighteen (18) compounds from various species of Aglaia (*A. ignea, A. duppereana, A. cucculata, A. euphoroides and A. tsangii*) showed only ignT1 (dammarenolic acid), dupT1 (aglaiol) and cucT1 (niloticin) exhibited selective anti-RSV activity. Time-of-addition studies revealed that both ignT1 and dupT1 inhibit RSV replication at a post-entry stage, with ignT1 being significantly more potent than dupT1. This post-entry inhibition of viral replication could suggest that the inhibitors possibly target the viral replicative enzyme, the RNA polymerase. The compound IgnT1 demonstrated favorable cellular safety when compared to reference plant derived diterpenoid compound (aphidicolin), which was at the concentration used about twice as cytotoxic as ignT1 while demonstrating virtually no anti-RSV activity. Besides, ribavarin which is the only currently approved anti-RSV therapeutic agent exhibits

much more toxicity resulting from its effect on cellular RNA and DNA polymerases (Lafeuillade *et al*. 2001; Prince 2001; Seetharama and Narayana 2005). Moreover, we also observed that methylation of ignT1 resulted in a complete loss of anti-RSV as well as cytotoxicity. This remarkable loss of activity could be related to an interaction of the polar carboxylic group of dammarenolic acid with a potential target molecule of the virus. In the case of the methylated derivative this group is chemically masked and hence the interaction is nullified. However, this hypothesis needs to be further confirmed. Thus anti-RSV compounds from *Aglaia species* present useful sources of lead compounds against RSV.

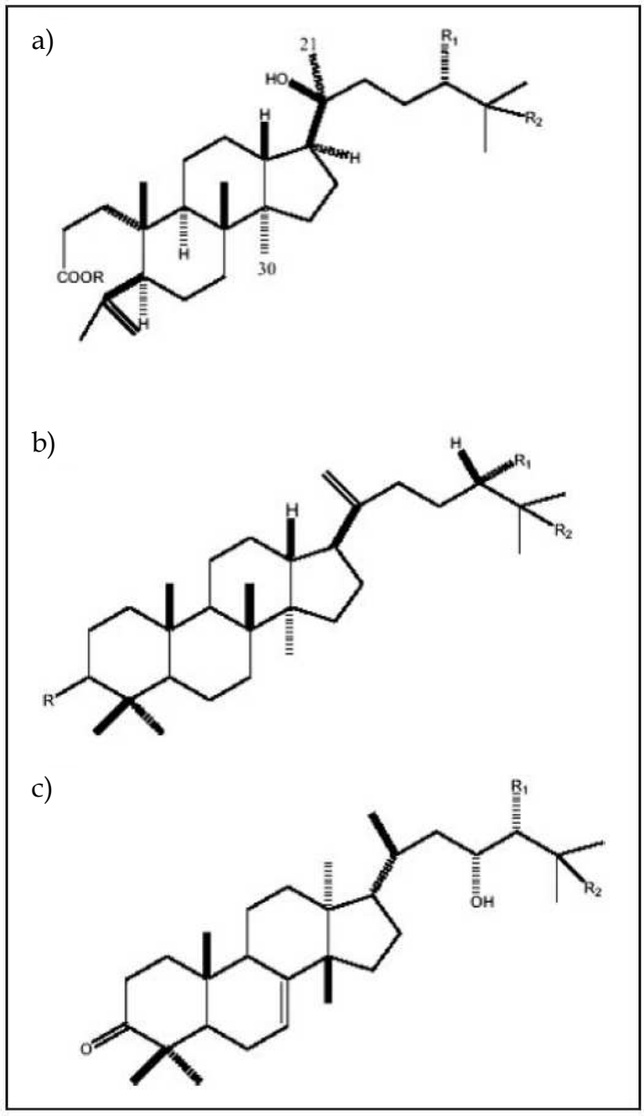
Code	Name	Isolated from	[a]IC_{50} [µg/ml]	[b]TC_{50} [µg/ml]	[c]S.I.
[ignT1]	Dammarenolic acid	*A. ignea* – bark	0.1	2.9	29
[ignT1A]	Methyldammarenolate	*A. ignea* – bark	>40	>40	[d]ND
[ignT2]	(20*S*,24*S*)-20,24-Dihydroxy-3,4-secodammara-4(28),25-diene-3-carboxylic acid	*A. ignea* – bark	>40	10.4	[d]ND
[ignT4]	(20*S*,23*E*)-20,25-Dihydroxy,3,4-secodammara-4(28),23-diene-carboxylic acid	*A. ignea* – bark	>40	70.4	[d]ND
[ignT3]	(23*E*)-(20*S*)-20-hydroxy-25-methoxy-3,4-secodammara-4(28),23-diene-3-carboxylic acid	*A. ignea* – bark	>40	68.4	[d]ND
[ignT5]	Methylester of 20*S*,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-4(28)-dammaren-3-carboxylic acid	*A. ignea* – bark	>40	14	[d]ND
[dupT4AB]	Mixture of epimers eichlerianic acid (24(*S*) [8a]) and shoreic acid (24(*R*) [8b])	*A. duppereana* – roots	39.6	12.3	0.3
[dupT5AB]	Mixture of epimers cabraleone (24(*S*)) and ocotillone (24(*R*))	*A. cucculata*- twigs	7.2	7.9	1.1
[dupT1]	Aglaiol	*A. duppereana* – leaves	11.8	168.8	14.3
[dupT2]	24,25-Epoxy-dammar-20-ene-3-one	*A. duppereana* – leaves	21.7	>40	[d]ND
[dupT3]	24,25-Dihydroxy-5α-dammar-20-ene-3-one	*A. duppereana* – leaves	25.5	52.1	2.0
[eupT1]	31-Nor-cycloartenol (29-nor-cycloartenol)	*Aglaia euphoroides* – leaves	>40	>40	[d]ND
[tsaT4]	4α,14-Dimethyl-9,19-cyclocholestan-3β,24α,25-triol	*Aglaia tsangii* – leaves	18.4	19.6	1.1
[tsaT3]	24,25-epoxy-cycloartan-3-ol	*Aglaia tsangii* – leaves	30.1	63.2	2.1
[cucT1]	Niloticin	*A. cucculata* – twigs	15.8	66.8	4.2
[cucT2]	Piscidinol A	*A. cucculata* – twigs	12.8	17.7	1.4
[tsaT1]	Lupeol	*Aglaia tsangii* – leaves	21.2	25.7	1.2
[tsaT2]	Lupeone	*Aglaia tsangii* – leaves	18	14.8	0.8

[a] Concentration of compound (µg/ml) that inhibits RSV infectivity by 50%
[b] Concentration of compound (µg/ml) that inhibits viability of target cells (HEp2) by 50%
[c] Selectivity index (SI) = TC_{50}/IC_{50}
[d] Not determined (ND), because observed activity was not dose-dependent
Experiments were performed at least thrice
Reproduced from Esimone *et al*., 2008

Table 1. Summary of anti-RSV screening of purified compounds from *Aglaia spp*

2.2 *Ramalina farinaceae*

Some lichens and lichen-derived substances have been shown to possess anti-viral activities (Cohen *et al*., 1996; Pengsuparp *et al*., 1995; Neamati *et al*., 1997). *Ramalina farinacea*, a lichen found to Nigeria but also available in other isolated places, has earlier been shown to possess broad anti-retro-viral (including lentiviruses) and anti-adenoviral principles (Esimone *et al*., 2005). Depsides and depsidones have been previously identified as antimicrobial active phytochemicals from *R. farinaceae* (Esimone and Adikwu, 1999; 2002; Esimone *et al*., 1999; 2006). In another related screening studies for active constituents against the respiratory syncytial virus, the ethylacetate fraction (*ET4*) of the plant was effective against RSV (IC_{50}= 3.65µg/ml). Mechanistic studies suggested that *ET4* targets an entry rather than a post-entry step by inhibiting the RSV fusion protein (Esimone *et al*., 2009). Further screening exercises and isolation studies are ongoing.



a) ignTl (Dammarenolic acid, isolated from the bark of *Aglaia ignea*)
b) dupTl (Aglaiol, isolated from the twigs of *Agliaia duppereana*)
c) cucTl (Niloticin, isolated from the twigs of *Aglaia cucculata*)
Reproduced from Esimone *et al*., 2008

Fig. 1. Structual formulae of anti-RSV compounds isolated from *Aglaia* spp.

2.3 Anemarrhena asphodeloides

The rhizomes of *Anemarrhena asphodeloides* Bunge (Liliaceae) have been used as a traditional medicine for anti-diabetic, anti-phlogistic, anti-pyretic, asthma, cough, bronchitis, allergy, sedative, diuretic, and anodyne properties in Korea, China, and Japan (Duke *et al*., 2002). Phytochemicals present in this species include xanthones (Pardo-Andreu *et al*., 2006), norlignans (Iida *et al*., 2000; Park *et al*., 2003; Lim *et al*., 2009), and steroidal saponins (Nakashima *et al*., 1993; Sy *et al*., 2008; Ren *et al*., 2006; Wang *et al*., 2010), associated with

biological activities such as anti-diabetic (Nakashima *et al*., 1993), anti-cancer (Sy *et al*., 2008), anti-oxidant (Pardo-Andreu *et al*., 2006), anti-fungal (Iida *et al*., 2000; Park *et al*., 2003), anti-depressant (Ren *et al*., 2006), anti-inflammatory (Lim *et al*., 2009) activity, and neuroprotective effects (Wang *et al*., 2010). Nyasol and its derivatives isolated from the ethyl acetate fraction of *A. asphodeloides* rhizomes had potent RSV inhibitory potential (IC_{50}= 0.39 to 0.89 μM) (Bae *et al*., 2007). Thus, these three known phenolic compounds, (-)-(R)-nyasol (= 4,4′-(1Z,3R)-Penta-1,4-diene-1,3 diyldiphenol; 1), its derivative (-)-(R)-4′-O-methylnyasol (2), and broussonin A (3) isolated from the rhizomes of *Anemarrhena asphodeloides* were for the first time identified as the active principles capable of efficient respiratory syncytial virus (RSV) inhibition. Later on, Youn and coworkers (Youn *et al*., 2011) working with this plant to find novel inhibitors of plant origin against the RSV-A2 strain propagated in HEp-2 cells, the butanol extract of the rhizomes of *A. asphodeloides* showed significant inhibitory activity. Two steroidal saponins and two xanthone derivatives were isolated from the butanol extract of the rhizomes of *A. asphodeloides*. The structures of the isolated compounds were identified as timosaponin A-III (1) (Kawasaki *et al*., 1963), anemarsaponin B (2) (Dong *et al*., 1991), mangiferin (3) (Qin *et al*., 2008), and neomangiferin (4) (Qin *et al*., 2008) using 1D- and 2D-NMR techniques such as 1H-13C HSQC and 1H-13C HMBC experiments and by comparison with published values. All the isolates (1-4) were evaluated for their ability to inhibit RSV. Timosaponin A-III exhibited potential anti-viral activity against the RSV-A2 strain propagated in HEp-2 cells, with an IC_{50} of 1.00 μM, which is more potent than the positive control, ribavirin (IC_{50} = 1.15 μM). The remaining compounds anemarsaponin B (2) which has a furostanol skeleton, and xanthone glycosides (3 and 4) were inactive (IC_{50} > 5 μM). These results suggest that the spirostane skeleton (1) is more active than the furostanol structure (2) in the steroidal saponins. They envisaged that further study with more diverse compounds is needed to develop these structure-activity relationships.

2.4 *Ligustrum lucidum*

From Ma Shuang-Cheng and coworkers (2001) report is made about activities of six secoiridoid glucoside compounds, lucidumoside C, oleoside dimethylester, neonuezhenide, oleuropein, ligustroside and lucidumoside A, isolated from the fruits of *Ligustrum lucidum* (Oleaceae). They were examined *in vitro* for their activities against four strains of pathogenic viruses, namely herpes simplex type 1 virus (HSV-1), influenza type A virus (Flu A), respiratory syncytial virus (RSV) and parainfluenza type 3 virus (Para 3) with Oleuropein being the most potent (IC_{50} 23.4μg/ml) as well as possessing an overall large and best therapeutic window (TC_{50} 562.5μg/ml/IC_{50} 23.4μg/ml) comparable to that of ribavirin, an approved drug for the treatment of RSV infections in human. Oleuropein is known to possess a wide range of biological activities (Ma *et al*., 2001), one of them being immune-modulatory activities (He *et a*l., 2001; Saija *et al*., 1998; Visioli *et al*., 1995; 1998;). The associated immune effects could enhance its anti-RSV benefit in the biological compartment.

2.5 *Hydroclathrus clathratus* and *Lobophora variegata*

Products from marine organisms show many interesting activities. Their constituents are more novel than those of many terrestrial plants. Seaweeds have long been recognized as rich and valuable natural resources of bioactive compounds because of their various biological properties (Mayer and Lehmann, 2000). The water-soluble extracts of seaweeds

have been shown to exhibit anti-viral activity against a wide spectrum of viruses (Witvrouw and de Clercq, 1997). There are more than 200 species of seaweeds in Hong Kong coastal waters (Ang, 2005), but research on their anti-viral activity is very limited (Zhu *et al*., 2003). Wang *et al*. (2008) in their study employed crude water extracts of six species of seaweeds *Colpomenia sinuosa* (Mertens ex Roth), *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *Hydroclathrus clathratus*, *Lobophora variegata* (Lamouroux) Womersley ex Oliveira, *Padina australis* Holmes and *Sargassum hemiphyllum* (Turner) C. Agardh from Hong Kong coastal waters were examined for their cellular toxicity and anti-viral activities. *H. clathratus* and *L. variegate* showed potent anti-RSV activities with EC_{50} values of 25µg/ml and 100µg/ml respectively.

2.6 *Echinacea purpurea*

Several viruses associated with upper respiratory diseases have been shown to stimulate the secretion of pro-inflammatory cytokines, including chemokines, sometimes in the absence of viral cytopathology. Some plant natural products are known to hold potential of reversing the pro-inflammatory effects induced by these viruses, and hence the disease-associated symptoms due to their infections (such as cold and flu symptoms of respiratory viruses). One such candidate agent is the herbal medicine *Echinacea purpurea*, which has become one of the most popular commercial herbal preparations in North America and Europe (Brevoort, 1998; Barnes *et al*., 2005). There have been numerous reports of immune modulatory properties in various preparations derived from different parts of several species of *Echinacea* (Gertsch *et al*., 2004; Barnes *et al*., 2005; Sharma *et al*., 2006, 2008; Wang *et al*., 2006), although the composition of these preparations is inconsistent, a fact that has made it difficult to propose a mechanism of action (Woelkart and Bauer, 2007). Widely varied anti-viral properties among different *Echinacea* species and component parts have been reported (Hudson et al., 2005; Vimalanathan *et al*., 2005). Thus it is important to carry out research on *Echinacea* preparations that have been standardized and chemically characterized.

Sharma *et al*., 2009 evaluated the ability of a standardized preparation of the popular herbal medicine *Echinacea* (Echinaforce®), an ethanol extract of herb and roots of *E. purpurea*, and containing known concentrations of marker compounds) to inhibit the viral induction of various cytokines in a line of human bronchial epithelial cells (BEAS-2B), and in two other human cell lines. They found *Echinacea* (Echinaforce® to inhibit respiratory syncytial virus (RSV)-induced IL-6 and IL-8 (CXCL8) secretion, in addition to several other chemokines. In every case however *Echinacea* inhibited this induction. The *Echinacea* preparation also showed substantial virucidal activity against RSV (MIC 2.5µg/ml) indicating the multi-functional potential of the herb. These results support the concept that certain *Echinacea* preparations can alleviate "cold and flu" symptoms, and possibly other respiratory disorders, by inhibiting viral growth and the secretion of pro-inflammatory cytokines.

2.7 *Dysoxylum gaudichaudianum*

The medicinal plant *Dysoxylum gaudichaudianum* Miq. (Meliaceae) is local to Papua New Guinea. The leaves and bark are used as a medicine by the indigenous people for treating rigid limbs, facial distortion in children, lumps under the skin, and other irritations, and as a remedy for sexually transmitted diseases (Weiner, 1984). They are also reportedly used as a remedy for fish poisoning and for convulsions (Cambie and Ash, 1994). A liquid drink made

by adding boiling water to the chopped leaves is considered to be a cure for most aches and pains (Parham, 1943) and is used for lung hemorrhage (Donald *et al*., 1975). Anti-RSV agents from *Dysoxylum gaudichaudianum* were described by Chen *et al*., 2007. Both aqueous (water or 1:1 water-2-propanol) and organic (1:1 methylene chloride-2-propanol) extracts of *D. gaudichaudianum* bark showed inhibitory activity against the RSV strain A2 in *CPE* inhibition and plaque reduction assays. Using respiratory syncytial viral CPE inhibition and plaque reduction assays to guide bioactivity-directed fractionation, the active fraction was found to be present in the more lipophilic phase, following liquid-liquid partition (chloroform/ aqueous ethanol). Reversed-phase chromatography of the organic extract led to the complete separation and isolation of four structurally related new compounds. The four new compounds, belonging to the tetranortriterpenoid family, named dysoxylins A-D, which were found to exhibit potent anti-viral activity against RSV. NMR spectroscopic analysis of this fraction indicated the presence of complex structures. These new compounds showed significant anti-RSV activity in both the cytopathic effect (CPE) inhibition and plaque reduction assays (1 to 4 μg/ml).

2.8 *Caesalpinia minax*

Caesalpinia minax Hance, a member of the *Caesalpinia* genus, is a prickly shrub growing in the tropics and subtropics. The seeds of this plant, which is called 'ku-shi-lian', have long been used as Chinese folk medicine for the treatment of common cold, fever and dysentery (Jiangsu New Medical College, 1977). Jiang *et al*. (2001) working with the chloroform fraction of the ethanol (95%) extract of the seeds was found to show *in vitro* anti-RSV activity, and a subsequent bioassay-guided study led to the isolation of a novel rearranged vouacapane diterpenoid possessing a new carbon skeleton, now designated spirocaesalmin. Spirocaesalmin, a novel rearranged vouacapane diterpenoid that exhibits significant activity against respiratory syncytial virus, possesses a new carbon skeleton with a spiro-CD ring system. Spirocaesalmin has been found to exhibit significant activity against respiratory syncytial virus (IC_{50} = 19.5 ± 1.5 μg mL, TC_{50} = 126.9 ± 2.0 μg ml and SI = 6.5) in cell culture, and the corresponding values for the positive control (ribavirin) are 3.6 ± 0.2 μg ml, 62.5 ± 1.9 μg ml and 17.4, respectively. Thus isolation of spirocaesalmin with a novel spiro-heterocyclic ring skeleton and the first bioassay against RSV in the family of vouacapane diterpenoids provide a potentially useful lead to the search for anti-viral drugs.

2.9 *Lithraea molleoides*, *Polygonum punctatum* and *Myrcianthes cisplatensis*

The search for anti-viral agents against RSV among the province of Entre Rı´os in Argentina has led to the discovery of plants which are expected to lead to identification of promising hits. These plants include *Lithraea molleoides*, *Polygonum punctatum* and *Myrcianthes cisplatensis*.

The trees of the genus *Lithraea* Hook. et Arn. (Anacardiaceae) are traditionally known for their irritating effects, especially among woodcutters and carpenters. *Lithraea molleoides* (Vell.) Engler called 'a´rbol malo' (evil tree), common name 'chichita', or 'molle de Co´rdoba', produces discomfort, drowsiness, lack of strength, rash and swelling in exposed parts of the body to anyone approaching to the tree. A myth exists about the healing powers of this tree, which also provides good quality timber and has tanning and dyeing bark (Munoz, 1990). Fruits have a volatile oil believed to cause, according to the ancient tradition, very strong and

disturbing irritation of the eyes and the skin (Storni, 1994). In the northwest of Argentina the fruits are used to make an alcoholic drink. The infusion of the leaves and fruits is said to be diuretic and stomachic (Cabrera, 1938). A decoction of the twigs is useful for breathing and digestive diseases (Ratera and Ratera, 1980). The tincture and the decoction are a good remedy for cough, bronchitis and phlegm. They are also hemostatic, stomachic, tonic and refreshing (Burgstaller Chiriani, 1974) and are used for arthritis (Martius, 1843).

The genus *Polygonum* L. is rich in medicinal species in the old as well as the new world. All of them are rich in tannins and some are occasionally used as foods (Lewis and Elvis-Lewis, 1977). *Polygonum punctatum* Elliot (Polygonaceae) is one of the most widely spread species of the genus in the province of Entre Rı´os, and is found in different habitats. It is considered poisonous to man and ocasionally fatal to livestock (Lewis and Elvis-Lewis, 1977). The acrid juice can cause both internal and external inflammation. It should be used only in professionally-made preparations and with medical supervision. It has astringent, diaphoretic, diuretic and rubefacient properties. A cold extract can be applied to skin problems, scabies, and hemorrhoids and as a gargle for toothache and problems in the larynx. The juice, pure or thinned with water, is effective in drawing pus out of sores (Lust, 1974). Infusion of the whole plant of *Polygonum punctatum* Elliot var., Aquatile (Martins) Fasset, also called 'erva do bicho' or 'caa-tai', 'ajicillo', is used in traditional medicine by the Toba indians of the northeastern region of Argentina, as a disinfectant and vulnerary in lavages of pimples, wounds and rash and as an antihemorrhoidal (Martı´nez Crovetto, 1964, 1965, 1981).

Many species of Argentine *Myrtaceae* are recognized as astringents and they are often mixed to potentiate their effects. The infusion of the leaves and the wood decoction of *Myrcianthes cisplatensis* (Camb.) Berg. (Myrtaceae) 'lapachillo', 'guayabo colorado' or 'palo pelado', is claimed to be astringent, tonic, stimulant, febrifuge and diuretic, and especially useful to wash and heal ulcers (Gonza´lez Torres, 1992). It is also a good remedy for lung and bronchial affections (Font Quer, 1988). It is a very common tree in the river-banks of the province of Entre Rı´os, easily recognizable by its clear bark and the strong odor of its leaves,which have contributed for its popularity.

The anti-RSV activities of *L. molleoides*, *P. punctatum* and *M. cisplatensis* extracts were reported by Kott *et al*., 1998. They reported ED_{50} values ranging from 78 to 120µg/ml. Their preliminary work therefore validated the continued traditional utilization of these plants as anti-viral remedies especially against RSV, and concluded on the need to further purify to isolate active compounds for further developments as anti-RSV agents.

2.10 Anti-RSV herbs commonly used in traditional Chinese medicines

Ma *et al*. (2002) described about 44 Chinese herbs commonly used in treatment of RSV disease. Traditional Chinese medicinal herbs have long been used as remedies against infectious diseases in China. Traditional medicines in the form of hot water extracts have been used orally for the treatment of various diseases. It is very likely that hot water extracts of some herbs would exhibit direct anti-viral activity *in vitro* at the concentration used for therapy. In this study Ma and coworkers assayed 44 medicinal herbs, which are currently used for the treatment of respiratory tract infectious diseases in China, to test anti-viral activities against RSV *in vitro* by cytopathic effect assay. The extracts of *Sophora flaescens* Ait.

and *Scutellaria baicalensis* Georgi with anti-viral properties were further investigated to identify their anti-viral components against RSV.

Aqueous extracts from these traditional Chinese medicines were also studied to detect anti-viral activity against RSV. Of all the 44 herbs tested, 41 showed anti-RSV activities with the following 25 herbs showing the strongest potency (IC_{50}= 6.3 to 52.1μg/ml) and also largest selective index (SI= 4.0 to 32.1): *Andrographis paniculata*, *Artemisia capillaries*, *Bupleurum chinense*, *Callicarpa nudiflora*, *Dendranthema morifolium*, *Forsythia suspensa*, *Ipomoea cairica*, *Gardenia jasminoides*, *Isatis indigotica*, *Lonicera japonica*, *Paeonia suffruticosa*, *Patrinia _illosa*, *Perilla frutescens*, *Phragmites communis*, *Platycodon grandiflorum*, *Polygonum cuspidatum*, *Polygonum multiflorum*, *Prunella _ulgaris*, *Pueraria lobata*, *Sarcandra glabra*, *Schizonepeta tenuifolia*, *Scutellaria baicalensis*, *Selaginella sinensis*, *Sophora flaescens* and *Tinospora* capillipes. All of these traditional Chinese medicines extracts were considered active, and of interest for further investigation. Further purification of 2 of the herbs (*Sophora flavescens* and *Scutellaria baicalensis*) led to the isolation of potent anti-viral compounds. *Sophora flaescens* and of *Scutellaria baicalensis* were further investigated and led to the identification from these two herbs anagyrine, oxymatrine, sophoranol, wogonin, and oroxylin A as the most potent anti-viral compounds against RSV; their IC_{50} values ranged from 7.4 to 14.5 μg/ml.

2.11 *Narcissus tazetta*, *Youngia japonica* and *Flos lonicerae*

Ooi *et al*., 2010 investigated the inhibitory effect of a novel mannose-binding lectin NTL isolated, purified and cloned from the bulbs of the Chinese daffodil, *Narcissus tazetta* var. *chinensis,* against human RSV, and various strains of influenza A (H1N1, H3N2, H5N1) and influenza B viruses. NTL was obtained after FPLC-gel filtration followed by desalting with a PD-10 column, and its purity was analysed by SDS-PAGE. It was determined to have a molecular mass of about 26 kDa by gel filtration and 13 kDa by SDS–PAGE. NTL is suggested to be a mannose-binding homodimer with two identical subunits of about 13 kDa. Molecular cloning revealed that the deduced amino acid sequence of the full-length cDNA encoding NTL contained a mature polypeptide consisting of 105 amino acids and a C-terminal peptide extension beyond the C-terminal amino acids Thr-Gly. NTL could effectively inhibit RSV-induced plaque formation (IC_{50}= 2.30 μg/ml). Its cytotoxicity against HEp-2 cells was low (CC_{50}= 325.4 μg/ml) and thus it had a high SI value of 141.36.

In another related earlier study, Ooi together with other investigators (Ooi et al., 2006) reported the anti-RSV activity of the ethanol extract of a biannual medicinal herb, *Youngia japonica* (commonly known as Oriental hawk's beard). Two potent anti-RSV compounds namely 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid, were subsequently purified and chemically characterized from the ethanol extract of *Youngia japonica*. The two dicaffeoylquinic acids exhibited prominent anti-RSV with an IC_{50} of 0.5μg/ml and there was no sign of cytotoxicity up to 100μg/ml concentration.

CJ 4-16-4 is a promising potent inhibitor of RSV isolated from *Flos lonicerae* using bioassay-guided fractionation. The drug inhibits of RSV in Hep-2 cells maintained in tissue culture at a very low concentration (~0.07 μM) with cell toxicity >400 μM (SI > 5880). In a cotton rat model of RSV infection, the drug was able to reduce viral titers by ~1 log at dose 12.5 and 25 mg/kg/day, and by >2 log at 100 mg/kg/day. This antiviral activity was specific as influenza A and B and herpes simplex 1 and 2 viruses were not inhibited (Ojwang *et al*., 2005).

Scientific name of Plant	Promising isolated compounds	References
Aglaia ignea	Dammarenolic acid	Esimone *et al*., 2008
Aglaia duppereana	Aglaiol	Esimone *et al*., 2008
Aglaia cucculata	Niloticin	Esimone *et al*., 2008
Anemarrhena asphodeloides	(-)-(R)-Nyasol* (-)-(R)-4′-O-methylnyasol* Broussonin A* timosaponin A-III*	Bae *et al*., 2007; Youn *et al*., 2011; Kawasaki *et al*., 1963
Dysoxylum gaudichaudianum	Dysoxylins A-D*	Chen *et al*., 2007
Sophora flavescens	Allmatrine Anagyrine Cytisine Isomatrine Matrine *N*-methylcytisine Oxymatrine Oxysophocarpine Sophocarpine Sophoranol Sophoridine	Ma *et al*., 2002
Scutellaria baicalensis	Wogonin Oroxylin A Baicalein Scutellarein Baicalin	Ma *et al*., 2002
Caesalpinia minax	Spirocaesalmin	Jiang *et al*., 2001
Ligustrum lucidum	Oleuropein	Ma *et al*., 2001
Narcissus tazetta	NTL	Ooi et al., 2010
Youngia japonica	Dicaffeoylquinic acids	Ooi et al., 2006
Flos lonicerae	CJ 4-16-4	Ojwang et al., 2005

Key: Promising isolated compounds determined on the basis of S.I index ≥ 4
*SI index not reported

Table 2. Promising anti-RSV compounds isolated from phytomedicines

3. Potential usefulness of anti-RSV compounds and phyto-constituents harnessed from phytomedicines in the treatment and control of RSV infection and disease

Complementary and alternative medicines have been used effectively by humans over several centuries for treating various diseases and can therefore be effectively employed to target the host response during RSV infection. Currently, no effective vaccine or therapeutic drug is available against RSV. Although ribavirin, a broad-spectrum anti-viral agent, is being marketed under approval, its clinical benefits are small and limited coupled, with the high cost and toxicity associated with it (Kneyber *et al*. 2000; Lafeuillade *et al*. 2001; Seetharama and Narayana 2005). New therapies designed to combat moderate to severe RSV infection and disease are clearly needed. Anti-RSV compounds from phytomedicines

could fill this gap. Although there have been several reports on natural anti-RSV agents, including some flavans (Li *et al*., 2006), caffeoylquinic acid (Li *et al*., 2005), and some alkaloids such as anagyrine, oxymatrine, and sophoranol (Ma *et al*., 2002). However, more anti-RSV drug candidates from phytomedicines need to be discovered for future development. A number of synthetic organic compounds have also received attention as anti-RSV agents however some are too cytotoxic to develop as clinically useful agents (Golankiewicz *et al*., 1995). Given the foregoing therefore, the potential usefulness of medicinal plants – either as suitable sources for the development of potent anti-viral anti-RSV agents or as effective tools in alternative medical practice, cannot be in question. Medicinal plants remain sources of cost-effective and accessible anti-viral remedies for use in developing and developed countries (Chen *et al*., 2008; Cowan, 1999; De Clercq, 1995; Jassim and Naji, 2003; Vlietinck and Vanden, 1991; Williams, 2001). They also could reduce time to be spent synthesizing new molecules. Moreover, reports of some strains of RSV developing resistance to currently administered therapeutic agents such as Ribavirin further underscore the need for effort for the development of new and more effective anti-viral agents to be undertaken. Finally, the screening of plants as a possible source of anti-viral in the ethnopharmacological approach enhances the probability of identifying new bioactive plant compounds (Vlietinck and Vanden, 1991; Baker *et al*., 1995). It is therefore hoped that anti-viral compounds from phytomedicines against RSV would greatly serve diverse usefulness in the management of RSV infection and disease.

4. Conclusion

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold in the development of new drugs: first, they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; second: a phytomedicine to be used for the treatment of disease. There are numerous illustrations of plant derived drugs. It is estimated that today, plant materials are present in, or have provided the models for at least 50% Western drugs (Schuster, 2001). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

Currently, investigations into the anti-RSV virus activities of numerous plant species are ongoing. A sense of urgency accompanies the search as the pace of species extinction continues. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened for currently. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles.

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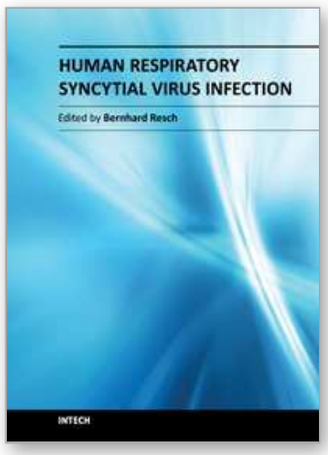

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In this online Open Access book on "Human RSV Infections", several distinguished authors contribute their experience in respiratory syncytial virology. A major focus lies on the fascinating pathophysiology of RSV and represents recent and actual work on different mechanisms involved in the complex pathogenesis of the virus. The second section elucidates epidemiologic and diagnostic aspects of RSV infection covering a more clinical view of RSV disease. At last, treatment modalities including the search for a vaccine that is still not in sight are discussed and conclude this book, thus building up a circle that runs from experimental models of RSV related lung disease over clinical aspects of disease to the latest news of therapeutic and prophylactic approaches to human RSV infection.

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