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SHORT COMMUNICATION

Potent bioactivities of the endemic Annonaceae heighten its dire conservation status

Aruna Weerasinghe[1], Shanmugam Puvenendran[1], Anura Wickramasinghe[1], D. Nedra Karunaratne[1], Siril Wijesundara[2] and Veranja Karunaratne[1]*

[1] *Department of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya.*
[2] *Royal Botanic Gardens, Peradeniya.*

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Abstract: Sri Lanka records 17 endemic species of the Annonaceae family. Worldwide, the Annonaceae are known to possess compounds with pharmaceutically important properties such as anticancer and insecticidal actions. In an attempt to investigate the mosquito larvicidal and antioxidant activities of the endemic Annonaceae plants, twelve plant species were collected. Of the 71 extracts investigated from various plant parts, five plants showed significant larvicidal activity with the CH_2Cl_2 extracts of the leaves of *G. hookeri* and *G. gardneri* (LC_{50} at 48 h = 0.4 and 0.3 ppm, respectively) exhibiting potency compared to the known larvicide (4S)-4-methyl-2-(11-dodecynyl)-2-butenolide (LC_{50}=0.3 ppm). Compared to the percent radical scavenging activity of the standard dl-α tocopherol (55.84), the MeOH extracts of the stem of *A. hortensis* (56.30), the leaves of *U. semecarpifolia* (57.33), and the seeds of *X. nigricans* (62.06) showed very promising activity. Significantly, it is recorded that two of the Sri Lankan endemic Annonaceae plants are extinct (*P. moonii* and *A. hortensis*) and the rest, except for *U. sphenocarpa*, are critically endangered, endangered, vulnerable or near threatened. These grim statistics highlight not only the urgency and the importance of biodiversity conservation of the endemic Annonaceae of Sri Lanka but also of investigating the plants for new phamacophores.

Keywords: Annonaceae, antioxidant activity, biodiversity conservation, endemic species, mosquito larvicidal activity.

INTRODUCTION

In an estimated number of the 270,000 species of vascular flora, 12 % are threatened (Walter & Gillett, 1998) and these plants are scattered in 369 families found in 200 countries. Because of the lack of information due to, either poor taxonomic knowledge or gaps in field work, the number could be much higher. When the sample assessed is the population within a species, or when there is genetic erosion among species, the situation becomes grimmer (Harper & Hawksworth, 1994; Gaston, 1996). In Sri Lanka, of the 3314 listed flowering plants, 455 are threatened with about 70 plants having become extinct by 1997 (Walter & Gillett, 1998).

The World Conservation Monitoring Center has designated Sri Lanka as a 'hotspot' in terms of rich biodiversity and threats faced (Caldecott *et al*., 1994). About 25 % of the flowering plant species are endemic to the island (Gunatilleke & Gunatilleke, 1990). The relict rainforest plant taxa of Gondwana-Deccan ancestry are now found only in some isolated forest pockets in Penninsular India and Southwestern Sri Lanka. The Deccan flora evolved in isolation in the late Cretaceous and early Tertiary periods, during the drifting of the Indian plate (Jayasekara, 1997). It has been hypothesized that biotic impoverishment, prior absence followed by colonization and later speciation has led to the tremendous endemic diversity in the island (Biswas, 2008). Except for a few reports (Bandara *et al*., 1989; Hewage *et al*., 1997; Hewage *et al*., 1998), there has not been a large-scale systematic search for bioactive agents from Sri Lankan flora so far. Although endemic plants such as *Salacia reticulata* var. *diandra* (Celastraceae) have underscored the potential of Sri Lankan plants (Gunatilaka *et al*., 1993; Dhanabalasingham *et al*., 1996; Yoshikawa *et al*., 1997), in general the bioactivity potential of Sri Lankan endemics remains relatively unknown. As such, there is an urgent need to investigate their therapeutic potential before they disappear forever.

* Corresponding author (veranjak@slintec.lk)

The Annonaceae are woody trees, shrubs and vines comprising about 130 genera and 2,300 species worldwide. Considering its large size, it is chemically one of the least known of the tropical plant families (Leboeuf *et al*.,1982). However, plants of the Annonaceae have received increased phytochemical and pharmacological attention in recent years; this is mostly due to the discovery of Annonaceous acetogenins, a class of natural products with a variety of biological activities (Cave *et al*., 1997; Kojima & Tanaka, 2009; Liaw *et al*., 2010). In Sri Lanka, Annonaceae is centered in the lowland rainforests (Dassanayake & Fosberg, 1980). They extend to the dry and lower montane zones, but are absent from elevations above 1500 m. Due to extensive deforestation in the humid regions, many species have become rare. In Sri Lanka, 17 endemic species, namely, *Desmos zeylanica* Hook. f. & Thoms., *Desmos elegans* (Thwaites) Saff., *Uvaria semecarpifolia* Hook. f. & Thoms., *Uvaria sphenocarpa* Hook. f. & Thoms., *Sageraea thwaitesii* Hook. f. & Thoms., *Phoenicanthus coriacea* (Thw.) H. Huber, *Phoenicanthus obliqua* (Hook. f. & Thoms.) Alston, *Alphonsea hortensis* H. Huber, *Polyalthia persicaefolia* (Hook. f. & Thoms.) Thw., *Polyalthia moonii* Thawaites, *Miliusa zeylanica* ex Hook. f. & Thoms., *Enicosanthum acuminata* (Thw.) Airy-Shaw, *Xylopia nigricans* Hook. f. & Thoms., *Goniothalamus gardneri* Hook. f. & Thoms., *Goniothalamus hookeri* Thw., *Goniothalamus thomsonii* and *Goniothalamus salicina* Hook. f. & Thoms., belonging to ten genera are recorded (Dassanayake & Fosberg, 1980), although many have not been collected for decades. It is in this backdrop, that the antioxidant and mosquito larvicidal activities of the extracts of 12 Annonacea belonging to 8 genera are reported in the present investigation. However, five endemic Annonaceae species could not be collected during plant collection between 2004 to 2010, which may have disappeared from their known habitats.

METHODS AND MATERIALS

Endemic Annonaceae plants were collected from Central Sri Lanka during 2004 - 2010 (Table 1) and identified and deposited at the National Herbarium of Royal Botanic Gardens, Peradeniya.

Air dried, ground plant materials (100 - 500 g) were extracted sequentially for 24 h at room temperature with CH_2Cl_2 followed by MeOH (500 - 1500 mL each) by using a bottle shaker. The combined extracts were concentrated in vacuo at 35 ^oC to obtain the respective crude extracts.

The mosquito larvicidal assay was carried out according to Ratnayake *et al*. (2001), with solutions of 0.5 mg/mL (500 ppm) of the plant extracts using second instar larvae of *Aedes aegypti*. The potent larvicide, (4S)-4-methyl-2-(11-dodecynyl)-2-butenolide was used as the postive control (Ratnayake *et al*., 2001). LC_{50} values at 48 h (mean ± SD) in ppm, were based on 4 concentrations and 4 replicates and were determined by using MiniTab statistical software.

Radical scavenging activity (antioxidant activity) of plant extracts against stable DPPH radicals was determined spectrophotometrically by the slightly modified methods of Miliauskas *et al*. (2004) and Yen and Duh (1994). dl-α tocopherol was used as the positive control. All the determinations were performed in 3 replicates and averaged.

RESULTS AND DISCUSSION

Out of the 71 extracts studied, 25 extracts showed toxicity against *A. aegypti* . The extracts of *G. gardneri* and *G. hookeri* demonstrated exceptionally high larvicidal activity while *A. hortensis, E. acuminata, X. championii, U. sphenocarpa* and *U. semecarpifolia* showed significant activity (Table 1). The most active was the dichloromethane leaf extracts of *G. hookeri* and *G. gardneri* (LC_{50} at 48 h = 0.4 and 0.3 ppm, respectively). Significantly, larvicidal potency of extracts of *G. hookeri* and *G. gardneri* were comparable with the potent larvicide (4*S*)-4-methyl-2-(11-dodecynyl)-2-butenolide (Ratnayake *et al*., 2001). Although no reports of either bioactivity or use in traditional medicine exists for these two plants in Sri Lanka, plants of the genus *Goniothalamus* are known for the presence of cytotoxic acetogenins and styryl-lactones (Leboeuf *et al*.,1982). However, of the large majority of the 166 *Goniothalamus* plants known globally, only 22 species have so far been investigated (Wiart, 2007).

Several extracts exhibited potent percent antioxidant activity. For example, MeOH extract of the stem of *A. hortensis* (56.30), the leaves of *U. semecarpifolia* (57.33), and the seeds of *X. nigricans* (62.06) showed higher radical scavenging activity compared to the standard dl-α-tocopherol (55.84). In recent times, free radicals have been implicated in inflammation processes, cardiovascular disease, rheumatoid arthritis, neurodegenerative disease, and the ageing process (Hollman & Katan, 1999). *X. nigricans, X. parvifolia* and *X. championii*, of which the latter two are non-endemic, are rich in isoquinoline alkaloids where some have shown potent antioxidant activity (Wijeratne *et al.,* 1996, 2001; Puvenendran *et al.,* 2008, 2010).

Table 1: Mosquito larvicidal activity and antioxidant activity of endemic Annonaceae of Sri Lanka

Genus Botanical name	Habit[a]	Locality	Habitat	Plant part(s)[b]	Type of extract(s)	% Mortality [c]	% AOA [e]
Alphonsea *A. hortensis* H. Huber	Tr	Kandy	Southwestern lowland	Lf	CH_2Cl_2	100 (114 ± 0.32)[d]	40.62 ± 0.24
				Lf	MeOH	35	32.81 ± 0.28
				Sd	MeOH	100	30.31 ± 0.04
				St	CH_2Cl_2	100 (46.9 ± 0.12)	13.69 ± 0.22
				St	MeOH	85	56.30 ± 0.05
				SBk	MeOH	100 (46.9 ± 0.6)	35.12 ± 0.24
Desmos *D. zeylanica* Hook. f. & Thoms	Tl	Kandy	Wettest parts of the lowland hill country at elevation between 200 and 800 m	Lf	CH_2Cl_2	100	2.49 ± 0.35
				Lf	MeOH	85	9.70 ± 0.50
				Sd	MeOH	100 (44.64 ± 0.08)	4.61 ± 0.06
				St	CH_2Cl_2	100	7.03 ± 0.12
				St	MeOH	95	5.84 ± 0.07
				SBk	MeOH	20	19.25 ± 0.12
Enicosanthum *E. acuminata* (Thw.) Airy-Shaw	Tr	Matale	Low elevation of both primary and secondary forests	Lf	CH_2Cl_2	100 (41.5 ± 0.14)	8.20 ± 0.16
				Lf	MeOH	100	36.52 ± 0.05
				St	CH_2Cl_2	100	13.52 ± 0.06
				St	MeOH	00	19.69 ± 0.25
				SBk	CH_2Cl_2	00	6.92 ± 0.10
				SBk	MeOH	100	45.59 ± 0.15
Goniothalamus *G. gardneri* Hook. f. & Thoms.	Tl	Kandy	Secondary and disturbed primary rainforests	Lf	CH_2Cl_2	100 (0.3 ± 0.34)	23.45 ± 0.32
				Lf	MeOH	100 (38.4 ± 0.86)	43.45 ± 0.14
				St	CH_2Cl_2	100 (1.4 ± 0.32)	12.45 ± 0.05
				St	MeOH	100 (5.3 ± 0.22)	14.65 ± 0.21
				Rt	MeOH	100 (3.7 ± 0.54)	26.63 ± 0.17
				SBk	CH_2Cl_2	100 (3.1 ± 0.04)	19.85 ± 0.31
				Rt	MeOH	100 (3.3 ± 0.82)	26.97 ± 0.52
				Fl	CH_2Cl_2	100 (29.5 ± 0.45)	14.74 ± 0.87
				Fl	MeOH	100 (14.4 ± 0.68)	17.91 ± 0.37
G. hookeri Thw.	Tr	Galle	Primary and secondary rainforests at low elevations	Lf	CH_2Cl_2	100 (0.4 ± 0.37)	4.50 ± 0.50
				Lf	MeOH	100 (45.6 ± 0.75)	14.03 ± 0.25
				SBk	CH_2Cl_2	100 (1.9 ± 0.12)	3.39 ± 0.35
				SBk	MeOH	100 (2.1 ± 0.64)	12.43 ± 0.93
G. salicina Hook. f. & Thoms.	Tl	Adam's Peak	Very local on hill sides and ridges in mixed dipterocarp forests	Lf	CH_2Cl_2	100 (4.5 ± 0.35)	10.97 ± 0.41
				Lf	MeOH	100 (38 ± 0.68)	59.98 ± 0.57
				St	CH_2Cl_2	80	9.79 ± 0.53
				St	MeOH	20	34.93 ± 0.65
				SBk	CH_2Cl_2	100	11.47 ± 0.21
				SBk	MeOH	00	40.85 ± 0.43
Phoenicanthus *P. coriacea* (Thw.) H. Huber	Tr	Kandy	An understorey tree of mid-mountain in forests along the Western and Southern edge of the main block	Lf	CH_2Cl_2	100 (132 ± 0.45)	13.21 ± 0.69
				Lf	MeOH	70	17.33 ± 0.81
				St	CH_2Cl_2	100	15.13 ± 0.23
				St	MeOH	00	35.95 ± 0.54
				SBk	CH_2Cl_2	60	13.84 ± 0.91
				SBk	MeOH	00	37.58 ± 0.47

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Genus Botanical name	Habit[a]	Locality	Habitat	Plant part(s)[b]	Type of extract(s)	% Mortality [c]	% AOA [e]
P. obliqua Hook. f. & Thoms.	Tr	Kandy	A tree in the South west of Sri Lanka	Lf	CH_2Cl_2	80	11.24 ± 0.78
				Lf	MeOH	00	20.98 ± 0.65
				St	CH_2Cl_2	100	14.56 ± 0.37
				St	MeOH	20	27.90 ± 0.54
Sageraea *Sageraea thwaitesii* Hook. f. & Thoms.	Tr	Nuwara Eliya	A tree found in the entire country	Lf	CH_2Cl_2	70	10.87 ± 0.65
				Lf	MeOH	20	40.64 ± 0.82
				St	CH_2Cl_2	80	32.96 ± 0.32
				St	MeOH	40	16.63 ± 0.43
Uvaria *U. sphenocarpa* Hook. f. & Thoms.	Cl	Kandy	Local in both dry and wet parts of the island at elevations between sea level and 720 m	Lf	CH_2Cl_2	70	6.08 ± 0.20
				Lf	MeOH	00	1.49 ± 0.06
				St	CH_2Cl_2	00	4.24 ± 0.10
				St	MeOH	00	42.93 ± 0.80
				SBk	CH_2Cl_2	70	3.52 ± 0.23
				SBk	MeOH	00	23.66 ± 0.17
U. semecarpifolia Hook. f. & Thoms.	Cl	Kegalle	Rather uncommon both in primary rainforests and secondary regrowth at elevations between 70 to 600 m	Lf	CH_2Cl_2	00	19.31 ± 0.20
				Lf	MeOH	00	57.33 ± 0.42
				SBk	CH_2Cl_2	65	33.23 ± 0.12
				SBk	MeOH	00	26.66 ± 0.60
X. nigricans Hook. f. & Thoms.	Tr	Matale	Rare tree of the dry and intermediate zones at low elevations	Lf	CH_2Cl_2	45	21.03 ± 0.39
				Lf	MeOH	00	33.92 ± 0.40
				Sd	CH_2Cl_2	50	14.33 ± 0.67
				Sd	MeOH	00	62.06 ± 0.33
				St+SBk	MeOH	100 (142.5 ± 0.81)	25.58 ± 0.43
				St+SBk	CH_2Cl_2	00	9.18 ± 0.32
X. championii Hook. f. & Thoms.	Tr	Kithulgala	Wet parts of the country in the intermediate zones	Lf	CH_2Cl_2	65	27.82 ± 0.35
				Lf	MeOH	55	48.45 ± 0.19
				S+SBk	CH_2Cl_2	100 (137.5 ± 0.75)	26.28 ± 0.04
				S+SBk	MeOH	100 (104.0 ± 0.42)	25.18 ± 0.6

[a] Tr, Tree; Tl, Treelet; Cl, Climber. [b] Lf, Leaf; Sd, Seed; St, Stem; SBk, Stembark; Rt, Root; Fl, Flower.

[c] Average of four replicates, each beaker containing five second instar larvae of *A. aegypti*; % Mortality at 48 h with 500 ppm solution of extract; Solutions with no extract was used as a negative control; (4*S*)-4-methyl-2-(11-dodecynyl)-2-butenolide, which gives 100 % mortality at 1 ppm was used as positive control.

[d] LC_{50} values stated as Mean ± SD, in ppm, were based on four concentrations and four replicates.

[e] Percentage antioxidant activity (AOA) is based on three replications and is stated as Mean ± SD. The absorbance of the DPPH radical without antioxidant (negaive control) and the reference compound *DL*-α-tocopherol (positive control) which exhibited activity at 55.84 ± 0.05 were also measured.

A species is considered nationally threatened when it is evaluated to be critically endangered (CR), endangered (EN), or vulnerable (V) (Walter & Gillett, 1998). Globally, it is estimated that 9.2 % of Annonaceae species are threatened (Walter & Gillett, 1998). In 1997, among the Sri Lankan Annonaceae, *P. perscicaefolia, P. moonii, A. hortensis, G. thomsonii, M. zeylanica, P. coriacea, P. obliqua, U. semecarpifolia* and *X. nigricans,* were considered indeterminate (Table 2), while *G. hookeri, S. thwaitesii* and *E. acuminata* were considered vulnerable. On the other hand, *D. elegans,*

Table 2: Endemic Annonaceae conservation status in 1997

Inditerminate	Vulnerable	Not threatened
P. perscicaefolia	*G. hookeri*	*D. elegans*
P. moonai	*S. thwaitesii*	*D. zeylanica*
A. hortensis	*E. acuminata*	*G. gardneri*
G. thomsonii		*G. salicina*
M. zeylanica		*U. sphenocarpa*
P. coriacea		
P. obliqua		
U. semecarpifolia		
X. nigricans		

Source : Walter and Gillett, 1998

its very promising potential as a source of plant medicines. However, it also highlights the grim conservation status of the endemic Annonaceae. If urgent remedial action is not undertaken towards their conservation, these plants will be well on their way towards extinction. This work also highlights the importance of screening the endemic flora of the South Asian region for potential drugs before they are threatened by deforestration and other anthropogenic factors.

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Table 3: Endemic Annonaceae conservation status in 2010

EX	CR	EN	V	NT	LC
A. hortensis	*P. perscicaefolia*	*G. salicina*	*D. zeylanica*	*D. elegans*	*U. sphenocarpa*
P. moonai		*M. zeylanica*	*G. gardneri*	*X. nigricans*	
		P. coriacea	*G. hookeri*	*U. semecarpifolia*	
			G. thomsonii	*P. obliqua*	
			S. thwaitesii		
			E. acuminata		

Source : Weerakoon & Wijesundara, 2012.
EX (Extinct); CE (Critically Endangered); E (Endangered); V (Vulnerable); NT (Near Threatened); LC (Least Concerned)

D. zeylanica, G. gardneri, G. salicina and *U. sphenocarpa* were considered not threatened. However, by 2011 (Weerakoon & Wijesundara, 2012), the situation had dramatically shifted (Table 3) with *P. moonii* being extinct and *A. hortensis* having become extinct in the wild; *P. perscicaefolia* becoming critically endangered; *G. salicina, M. zeylanica* and *P. coriacea* considered endangered*; D. zeylanica, E. acuminata, G. gardneri, G. hookeri, G. thomsonii* and *S. thwaitesii* being vulnerable; *D. elegans, X. nigricans, U. semecarpifolia* and *P. obliqua* becoming near threatened. The only species apparently out of danger is *U. sphenocarpa*. Thus, the majority of the 17 endemic Annonaceae species are nationally threatened and one is extinct. This dire situation far exceeds the global average (9.2 %) of threatened species calling attention to a crisis situation. During the collection attempts, *D. elegans, P. perscicaefolia, G. thomsonii, P. moonii* and *M. zeylanica* could not be located due to their disappearance from recorded sites.

In conclusion, this study reveals the richness of bioactivity of the endemic Annonaceae of Sri Lanka, and

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