

ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF LEAF, TWIG AND CALLI EXTRACTS OF *Neolamarckia cadamba* (Roxb.) Bosser

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INTRODUCTION

Neolamarckia cadamba (Roxb.) Bosser (“Bakmee” S., “Wild cinchona” E.) is an important medicinal plant used in traditional medicine for the treatment of fever, uterine diseases, skin diseases, dysentery and diabetes (Dubey *et al.*, 2011). Large scale destructive collection of plant parts for herbal formulations and research purposes possibly pose a potential threat of extinction on this plant. The production of secondary metabolites is influenced by various environmental conditions (Okudera and Ito, 2009) and therefore, calli can be subjected to varying conditions for desired secondary metabolites.

Studies have been carried out on phytochemical analysis (Dubey *et al.*, 2011), antioxidant activity of leaf and bark (Alekhya *et al.*, 2013; Ganjewala *et al.*, 2013), antifungal activity of leaf and bark (Patel *et al.*, 2011), antibacterial activity of fruit (Mishra, 2011), antidiabetic property of leaf, hypoglycemic activity of leaf (Ahmed *et al.*, 2011) of *N. cadamba*. However, attempts to raise callus of *N. cadamba* explants for secondary metabolites is not extensively studied.

The objective of the present study is to explore the possibility of deducing whether the calli raised from *N. cadamba* can be used as a potential source for extraction of secondary metabolites instead of destructive sampling. The present study reports a comparison of antimicrobial and antioxidant potential of natural plant extracts of leaves and twigs of *N. cadamba*, and calli derived from leaf and internode explants of *N. cadamba*.

MATERIALS AND METHODS

Leaf and twig samples of *N. cadamba* were collected from Thalgahawila, Horana in the District of Kalutara, Sri Lanka between February to May 2014 and samples were identified by comparing herbarium specimens deposited at Herbarium, Peradeniya Botanical Gardens and related literature.

a) Calli Induction

Tender leaves emerging from axillary buds and internodes used for calli induction were washed and the surface sterilized using 0.05% HgCl₂ solution for 7 minutes 70% alcohol for 30 seconds and in warm sterile distilled water three times before placing on the culture medium (Indu *et al.*, 2013). A series of ten MS culture media of MS medium (20.0 ml) each with varying NAA and BAP hormone concentrations (NAA:BAP- 0.5:5.0/ 1.0:4.5/ 1.5:4.0/ 2.0:3.5/ 2.5:3.0/ 3.0:2.5/ 3.5:2.0/ 4.0:1.5/ 4.5:1.0 and 5.0:0.5) along with 3 mg/l of activated charcoal was used for calli generation. Ten explants were tested with each hormonal treatment.

b) Crude extraction

Air dried plant tissues (leaves and twigs) (10 g) and air dried calli tissue (three leaf-calli and three internode-calli) (4.5 g) were separately macerated and extracted into cold methanol and dried in a vacuum to a constant dry weight.

c) Bioassays

Antibacterial assay

Antibacterial activity against *Bacillus* sp., *Micrococcus* sp., *E. coli* and *Salmonella typhi* was deduce using standard well diffusion method using DMSO (Dimethyl sulfoxide) as the solvent. Extreme precautions were used when handling *Salmonella typhi* collected from the Durdans Hospital, Colombo. The bacterial suspension was prepared to 0.5 McFarland standards. Crude extracts (50 µg/µl) of leaf, twig, leaf-calli and internode-calli of *N. cadamba* were separately used for antibacterial assays. Gentamycin (2.6 µg/µl) was used as the positive control and 100% DMSO as the negative control (Dubey *et al.*, 2011).

Antifungal assay

Antifungal activity against *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp. and *Candida* sp., were determined using standard TLC plate method with a fungal spore suspension of 0.1 OD (measured

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at 550 nm). Crude extracts (50 µg/µl) of leaf, twig, leaf-calli and internode-calli of *N. cadamba* were separately used for antifungal assays. DMSO was used as the solvent. Amphotericin B of 5 µg/µl was used as the positive control and 100% DMSO as the negative control (Wadhawa *et al.*, 2013).

Antioxidant assay

Antioxidant assay of leaves and twigs of *N. cadamba* and calli extracts were tested by measuring the total phenolic content using FolinCiocaltau Phenol Reagent and DPPH radical scavenging method.

i) Total Phenol Content (TPC)

Crude extracts (1.0 g) of leaf, twig, leaf-calli and internode-calli were separately added to distilled water (0.9 ml) followed by adding FolinCiocaltau Reagent (0.5 ml), and 20% sodium carbonate (1.5 ml). The final volume of the mixture was made up to 10 ml with distilled water. Absorbance of mixture was measured at 750 nm. Phenolic content of extracts was calculated as gallic acid equivalents (GAE) in mg/g on the basis of standard curve of gallic acid (Chandel *et al.*, 2012).

ii) DPPH Radical scavenging activity

This method is for evaluating the ability of antioxidants to scavenge free radicals. Different concentrations of extracts (20, 40, 60 and 80 µg/ml) of leaf, twig, leaf-calli and internode-calli were dissolved in methanol and taken in test tubes in triplicates. Then 2.0 ml of 0.1 mM methanol solution of DPPH was added to each test tube and shaken vigorously. After keeping in dark for 30 minutes, absorption was measured at 517 nm. Results were compared with standard compound rutin (IC₅₀ = 54.05 µg/ml). Radical scavenging activity (RSA) % was calculated as follows (Chandel *et al.*, 2012).

RSA (%) = $\frac{Ab_{control} - Ab_{sample}}{Ab_{control}} \times 100$ (Chandel *et al.*, 2012).

Ab_{control} - Absorbance of DPPH in methanol, Ab_{sample} - Absorbance of a DPPH solution with the test sample. Statistical analyses of data were performed on the statistical software package SPSS PC Version 20.

RESULTS AND DISCUSSION

Table 1. Antibacterial activity of extracts of *N. cadamba* at 50 µg/µl

	Positive control	Negative control	Twig/internode extract			
	Gentamycin	DMSO	Callus	Natural	Callus	
<i>Micrococcus</i> sp.	30.03 (0.05)b	0.00	6.13 (0.15)a	4.93 (0.06)b	6.00 (0.10)a	4.83 (0.06)b
<i>E-coli</i>	26.05 (0.05)b	0.00	6.50 (0.10)a	5.10 (0.10)c	7.13 (0.12)b	5.33 (0.06)c
<i>Bacillus</i> sp.	28.02 (0.04)b	0.00	0.00	0.00	0.00	0.00
<i>Salmonella typhi</i>	28.00 (0.06)b	0.00	5.53 (0.06)a	4.73 (0.06)b	5.67 (0.12)a	4.73 (0.06)b

* Mean with standard deviation in parenthesis of triplicate analysis

Different letters across the rows indicate that no significant difference at $p \leq 0.05$.



(a)

(b)

Figure 1. Anti-bacterial assays for crude extracts of natural plant (leaf and twig) of *N. cadamba* against

(a) *Micrococcus* sp. And
(b) *Salmonella typhi* (well diameter = 4.5 mm)
(1-positive control, 2-negative control, 3-leaf extract, 4-twig extract)

The following hormone combinations used in MS medium were able to induce and develop calli from leaf and internode explants of *N. cadamba*.

Leaf - NAA 5.0mg/l : BAP 0.5mg/l and NAA 2.5mg/l : BAP 3.0mg/l (60% calli development)

Internode - NAA 5.0mg/l : BAP 0.5mg/l and NAA 2.5mg/l : BAP 3.0mg/l (70% calli development)

The calli extracts of leaves and internodes showed positive antibacterial activity (Figure 1) but comparatively lesser degree than that of the extracts of natural plant parts (Table 1). According to

Wadhawa *et al.* (2013), the highest activity was against *E. coli*, *Bacillus* and *Staphylococcus* sp. for methanolic extracts when a maximum of 75 µl/ml of *N. cadamba* leaf sample was used but in the present study antibacterial activity was not shown against *Bacillus* spp. at 50 µg/µl. If a higher concentration of the sample was used positive results could have been observed for *Bacillus* sp.

Methanolic extracts of leaves and twigs showed antifungal activity. Antifungal activity of natural twig extract (Figure 2 and Table 2) against *Trichoderma* spp. was considerably higher [11.17 mm] and lowest against *Candida* spp. [5.3 mm]. This supports the use of paste of crushed plant parts as a cure for skin diseases by ancient tribal communities. No antifungal activity of methanolic crude calli extracts of leaves and internodes was observed. Leaf extracts showed the highest activity against *Aspergillus fumigatus* (Wadhawa *et al.*, 2013) compared to *Candida albicans*. In the present study also, the highest antifungal activity was against *Aspergillus* sp. Patel *et al.* (2011) observed that the leaf extract possesses considerably higher activity than that of the bark extract, whereas in the present study the twig extract has shown better antifungal activity than that of the leaf extract.

Table 2. Antifungal activity of extracts at 50 µg/µl of extracts of *N. cadamba*

Microorganism	Zone of inhibition (mm)*				
	Positive Amphotericin B	Negative DMSO	Leaf	Twig	Leaf/Internode calli
<i>Penicilium</i> sp.	15.07 (0.06)a	0.00	6.07 (0.06)b	9.03 (0.06)c	0.00
<i>Aspergillus</i> sp.	14.10 (0.10)a	0.00	11.63 (0.23)c	10.03 (0.06)c	0.00
<i>Trichoderma</i> sp.	19.03 (0.06)a	0.00	9.07 (0.06)c	11.17 (0.15)d	0.00
<i>Candida</i> sp.	13.47 (0.06)a	0.00	5.47 (0.06)b	5.30 (0.00)b	0.00

* Mean with standard deviation in parenthesis of triplicate analysis

Different letters across the rows indicate that no significant difference at $p \leq 0.05$.

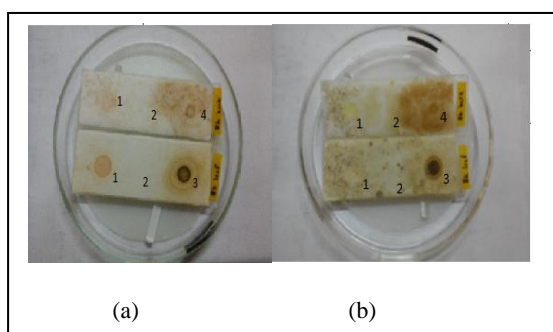


Figure 2. Anti-fungal assays for crude of natural plant (leaf and twig) extracts of *N. cadamba* against (a) *Aspergillus* sp. and (b) *Trichoderma* sp. (spot diameter= 6.0 mm) (1-positive control, 2-negative control, 3-leaf extract, 4-twig extract)

Total phenolic content (TPC) (mg/g GAE) was highest in crude (1.0 g) extracts of twigs (17.0) and leaf (4.0) compared to that of internode-calli (3.5) and leaf-calli (2.0) (Table 3). Radical scavenging activity (RSA) % increased with concentration of extracts. In RSA of extracts of *N. cadamba*, activity decreased in the order of twig, leaf, internode-calli and leaf-calli (Table 3), confirming the findings of Ganjewala *et al.* (2013). TPC and RSA values showed a similar trend in twig, leaf, Internode-calli and leaf-calli.

CONCLUSIONS/RECOMMENDATIONS

The results of the present study revealed that calli development was successful in *N. cadamba* (Roxb.) Bosser. The most effective hormonal combinations for calli formation were NAA 5.0mg/l:BAP 0.5mg/l and NAA 2.5mg/l:BAP 3.0 mg/l in MS medium for leaf-calli and internode-calli of *N. cadamba*. Although extracts of leaf-calli and internode-calli demonstrated positive antibacterial and antioxidant activities, they are less and reveal that secondary metabolites responsible for such properties are produced in calli but to a lesser extent. However, natural environment imposes various conditions of stress on naturally growing plants stimulating to produce more secondary metabolites in higher concentrations. This could be the reason, why leaf and twig extracts showed higher antimicrobial and antioxidant activity compared to that of calli extracts. As suggested in the literature (Indu *et al.*, 2013) if calli are subjected to optimization with stresses such

as salinity, water stress, electric stimulation, etc., there may be a possibility of production of secondary metabolites is higher in concentrations. The present study reveals that development of calli from leaves and internodes of *N. cadamba* could be considered as a potential source of bio-active compounds against some selected pathogenic and non-pathogenic bacteria and antioxidants to act against free radicals.

Table 3. Total Phenolic content (TPC) and Radical Scavenging activity (RSA) for different concentrations of leaf, twig and calli crude extracts of *N. cadamba*

Plant Part	TPC* (mg/g GAE) (SD)	RSA (%)* (SD) based on IC ₅₀ values for different concentrations (µg/ml) of extracts	
		20 µg/ml	40 µg/ml
Leaf-calli	2.0 (0.00)	11.00 (0.58)	20.67 (1.00)
		30.33 (1.16)	81.33 (0.58)
		19.00 (1.00)	59.67 (0.58)
		85.67 (2.08)	94.33 (0.58)
Internode-calli	3.5 (0.00)	72.67 (6.43)	69.67 (0.58)
		86.33 (2.31)	95.00 (1.73)
		61.67 (0.58)	81.33 (1.16)
		90.67 (0.58)	98.00 (0.00)
Leaf	4.0 (0.00)	61.67 (0.58)	81.33 (1.16)
		90.67 (0.58)	98.00 (0.00)
		61.67 (0.58)	81.33 (1.16)
		90.67 (0.58)	98.00 (0.00)
Twig	17.0 (4.58)	61.67 (0.58)	81.33 (1.16)
		90.67 (0.58)	98.00 (0.00)
		61.67 (0.58)	81.33 (1.16)
		90.67 (0.58)	98.00 (0.00)

*Mean with standard deviation in parenthesis of triplicate analysis

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