Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 3 March 2022

Research Article

Determination of the Antimicrobial Activity of Selected Medicinal Plant Species in the *Rubiaceae* Family

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Received: December 31, 2021

Published: February 23, 2022

Abstract

The discovery of novel antimicrobial agents has become one of the most important aspects of today's world. Sri Lanka has a diverse collection of medicinal plants that play an important therapeutic role for many decades. This study was designed to identify such indigenous plants from the Rubiaceae family and investigate their unknown antimicrobial activities. Based on their ayurvedic value and the scarcity of scientific research, five Rubiaceae plant species (*Knoxia zeylanica, Ophiorrhiza mungos, Oldenlandia herbacea, Oldenlandia herbacea, Wendlandia bicuspidata,* and *Morinda umbellata*) were selected. The antifungal activity of crude plant extracts was tested using the poison food technique against *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. fungal strains. Out of three fungal strains, *Penicillium* spp. showed the highest susceptibility to crude extracts; *K. zeylanica* (root), *O. mungos* (whole plant), and *O. herbacea* (stem and leaves). At a concentration of 2 mg/ml, the crude extract of *O. mungos* (whole plant) inhibited (98.56 %) the growth of *Aspergillus* spp., indicating the presence of strong antifungal compound/s in *O. mungos* species. The agar well diffusion method was used to conduct an antibacterial assay against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25928), *Bacillus cereus* (ATCC 11778), and two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 35218). The crude extract (1mg per well) of *K. zeylanica* (root) demonstrated the highest antibacterial activity against *B. cereus* (16.2 ± 0.3 mm) and *S. aureus* (16.9 ± 0.3 mm). According to the findings of this study, *K. zeylanica* and *O. mungos* could be potential candidate plants for further research to isolate both anti-bacterial and antifungal compounds.

Keywords: Medicinal Plants; Rubiaceae; Anti-fungal; Anti-bacterial; Ayurvedic Medicine

Abbreviations

SE: Standard Error; MHA: Muller Hinton Agar; PDA: Potato Dextrose Agar

Introduction

The emergence of drug-resistant microbes has become one of the biggest problems that we face as a community, and it has made us vulnerable to many pathogenic diseases [1]. Thus, the pharmaceutical sector is facing a huge challenge in developing new antimicrobial drugs more than ever [2]. As a result, a broader approach to solve this problem is more desired than ever before [3]. One such approach is the discovery of previously unknown secondary metabolites with antimicrobial properties in natural products. After identifying, validating, and developing potential targets, new antimicrobial agents against resistant species can be revealed. Sri Lanka has a diverse collection of medicinal plants that have been used to treat a variety of diseases in Sri Lankan traditional Ayurvedic medicine [4]. There is a high chance of having bioactive compounds in those plants, and this study was carried out to identify such plants belonging to the family Rubiaceae, to investigate their unidentified antimicrobial activities. Therapeutic information on plants in the Rubiaceae family was gathered from various Ayurvedic resources. In many countries, Rubiaceae species are used in folk medicine to treat a variety of diseases such as malaria, eczema, hepatitis, edema,

hypertension, cough, sexual weakness, and diabetes. These medical preparations are made from plant parts such as, leaves, roots, bark, and fruits. In some cases, the entire plant is used, including root. These can be used alone or in combination with other plants or materials of animal origin or mineral origin. Powder, decoction, and concoction are the most common forms of medicinal remedies [5].

In this study, we have selected five plant species [*Knoxia zeylanica* (stem and leaves together), *Knoxia zeylanica* (root), *Ophiorrhiza mungos* (whole plant), *Oldenlandia herbacea* (stem and leaves together), *Oldenlandia herbacea* (root), *Wendlandia bicuspidata* (leaves), and *Morinda umbellata* (whole pant)] belonging to the Rubiaceae family which have valuable traditional medicinal properties to determine their undisclosed antimicrobial activities. In Sri Lankan traditional medicine, *K. zeylanica* (Ela ratmal) is often used to treat inflammation caused by snake bites [4]. From *K. zeylanica* it is reported that triterpene, Ursolic acid has been isolated [6] and antibacterial activity was discovered in the stem and leaves of *K. zeylanica* [4]. *M. umbellata*, (Kiriwel) is used to treat bone fractures in Sri Lankan traditional medicine [4]. Phytochemical screening of the aqueous and ethanol extracts of *M. umbellata* has revealed the presence of alkaloids, saponins, flavonoids, glycosides, and phenols [7]. *M. umbellata* is being reported for significant anti-inflammatory and antioxidant activity [7], antibacterial activity against *S. aureus* bacterial strain [4], and cytotoxic activity against cancer cell lines *in vitro* [8]. *O. herbacea* (Wal kottamalli) is reported as one of the very valuable ayurvedic medicinal plants used in Sri Lanka. It has been used to treat many diseases including parasitic infections, diabetes mellitus, menstrual cycle disorders, nasopharyngeal infections, ear and eye diseases, vomiting, leprosy, stomach disorders, liver diseases, and venereal diseases [4]. Antibacterial activity is reported in the root, stem, and leaves in this plant [4]. Phytochemical screening of the *O. herbacea* has discovered the presence of Tannins, Glycosides, Saponins, Phenols, Alkaloids, Carbohydrates [9]. Rabies, cancers, and diarrhea are being treated in Sri Lankan traditional medicine using remedies extracted from *O. mungos* [4]. Different plant parts of this plant are reported to have antioxidant activity and possess high cytotoxic activity [10]. Also, *O. mungos* leave exhibits antibacterial activity against *S. aureus* Gram-positive bacterial strain [4]. Camptothecins, Glucosides, and Ergosterols are some key chemical constituents that have been reported in *O. mungos* species. *W. bicuspidata* (Rawana Idala) is reported to exhibit activity against *S. cerevisiae* a yeast species [11] and *S. aureus* bacterial strain [4]. A scandoside methyl ester, iridoid glycoside, has been isolated and reported from the wood of *W. bicuspidata* [12]. *W. bicuspidata* is used in treatment of dysentery, fever, diarrhea, ulcers, and rheumatism in Sri Lankan folk medicine [4].

In previous studies reports antibacterial properties of many plants that we selected [4,7,10-12]. Hence, in this study, we primarily focused to unravel the antifungal activity of crude plant extracts of selected plant species which were not tested previously. Antifungal activity was tested using the poison food technique against fungal strains of *Aspergillus* spp., *Rhizopus* spp., *and Penicillium* spp. Except *K. zeylanica* (root). Also in the current study antibacterial properties were tested suing a sequential extraction protocol (using Hexane, CH_2Cl_2, EtOAc, and MeOH) to identify the target antibacterial fraction/s in *O. herbacea* (root) and *W. bicuspidata* (leaves) among four fractions. As part of further investigation, the identified bioactive fraction (s) can be subjected to secondary metabolite identification.

Materials and Methods

Plant material

Plant materials were collected from different areas in Sri Lanka (Hadigalla, Godamunna, Mathugama, Neluwa, and Hirana) upon their availability. Based on their Ayurvedic value, different plant parts (roots, whole plant, leaves) of five Rubiaceae plants were collected. Specimens made of these collected plant species were authenticated at the National Herbarium of the Royal Botanical Garden, Peradeniya, and Bandaranayake Memorial Ayurvedic Research Institute, Maharagama. The voucher specimens used in studies were deposited in the herbarium of the Department of Plant Sciences, University of Colombo, Sri Lanka.

Small scale extraction

Fresh plant parts (roots, leaves, whole plant) were separately washed with water, air-dried at room temperature for about 1 week, and then pulverised using a domestic grinder. Each pulverised sample (a portion of 50g) was mixed with the 200 ml solvent mixture, dichloromethane, and methanol (1:1, v/v). The solvent-sample mixture was sonicated for 45 minutes. The mixture was filtered through Whatman No. 01 filter paper. The extraction procedure was repeated two times to ensure the complete extraction of bioactive compounds. The obtained filtrates were combined

and concentrated at 35°C using a rotary evaporator. The crude extract was further dried, and the dry weight was recorded [4].

Large scale extraction

O. herbacea whole plant powder (500g) and *W. bicuspidata* leaf powder (500g) were separately obtained. Each plant material was exhaustively extracted for 30 minutes in 1 liter of hexane using sonication. Extraction with the same solvent was repeated twice, and the filtrates were combined. The filtrate was evaporated under reduced pressure, maintaining a temperature below 40 °C using a rotary evaporator. The crude hexane extract was further dried, and the dry weight was recorded. Adopting the same protocol, the remaining plant material from the hexane extraction was dried and sequentially extracted from dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and methanol (MeOH).

Antimicrobial assays

Agar well diffusion assay for crude extracts

Antibacterial activity of the crude extracts of large-scale sequential extractions (Hexane, CH_2Cl_2, EtOAc and MeOH) of *O. herbacea* (whole plant) and *W. bicuspidata* (leaves) and the crude extract of small-scale extraction (CH_2Cl_2 and MeOH, 1:1, v/v) of *K. zeylanica* (root) was evaluated against *Staphylococcus aureus* (ATCC 25928), *Bacillus cereus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 35218). Dried Muller Hinton Agar (MHA) plates (Hardy, USA; 38.0g in 1000 ml of distilled water) were inoculated with 200 µl standardized inoculum (turbidity equivalent to 0.5 McFarland standard) of the bacterial cell suspension. The cell suspension was evenly distributed on the agar surface. For the preparation of bacterial cell suspension, a small inoculum of freshly grown bacterial culture (obtained after overnight incubation of bacteria-streaked MH agar plate at 37° C which was cultured using a bacteria culture stock) was taken with a sterile loop and dissolved in sterile saline water. Holes were made as required on the microbial inoculated agar plates using a sterile tip. A portion of dried crude extract was dissolved in the relevant solvent/ solvent mixture to reach a final concentration of 10 mg ml^{-1}. 100 µL of the sample was delivered onto a well to obtain 1 mg/well. A volume of 100 µl from the relevant solvent/solvent mixture was used as the negative control and Gentamycin (50 µg/well) was used as the positive control. [13,14].

Poison food technique for crude extracts

The antifungal activity of crude extract of small-scale extraction (CH_2Cl_2 and MeOH, 1:1, v/v) of *K. zeylanica* (stem and leaves together), *K. zeylanica* (root), *W. bicuspidata* (leaves), *M. umbellata* (whole plant), *O. herbacea* (stem and leaves together), *O. herbacea* (root) and *O. mungos* (whole plant) was evaluated against *Aspergillus* spp., *Rhizopus* spp. *and Penicillium* spp. The dried crude extract (20 mg in 1 ml of solvent) was added to the potato dextrose agar (PDA) liquid medium (19 ml of Hardy, USA; 38.0 g in 1000 ml of distilled water) before solidification occurred to reach the final concentration of the extract in a PDA plate at 1 mg/ml. A CH_2Cl_2 and MeOH mixture (1:1, v/v, 1 mL) was used as the negative control, and Nystatin was used as the positive control. PDA media containing crude extracts, negative control, and positive control were pre-incubated overnight. The next day, the inoculation was done using a small mycelia piece (around 3mm in diameter) harvested from an active fungi species and placed in the center of the PDA plate. Inoculated PDA plates were further incubated at Room Temperature. For *Rhizopus* spp., 3 days, and for *Penicillium* spp., and *Aspergillus* spp., 7 days of incubation periods were done. The antifungal activity of the test samples is calculated using equation 1 by comparing the colony diameter of the test sample plate with that of the reference plate (negative control). In Equation 1, D_c is the diameter of growth in the control plate (a negative control with the respective solvent) and D_s is the diameter of growth in the sample plate (with the plant extract) [15].

$$\text{Antifungal activity (\%)} = \frac{D_c - D_s}{D_c} \times 100$$

Equation 1: Antifungal effect of the test sample.

Inhibitory effect of *Ophiorrhiza mungos* (leaves) extract against *Aspergillus* spp.

A series of concentration values of *O. mungos* (leaves) crude extracts using CH_2Cl_2 and MeOH (1:1, v/v) solvent mixture was prepared using the following procedure. Dried crude extracts of *O. mungos* (leaves) were added into separate portions of PDA liquid medium (Hardy, USA; 38.0 g in 1000 ml of distilled water) before solidification occurred to reach the final concentration of the extract in the PDA plates, which had 2 $mgml^{-1}$, 1.75 $mgml^{-1}$, 1.50 $mgml^{-1}$, 1.00 $mgml^{-1}$, 0.75 $mgml^{-1}$, and 0.5 $mgml^{-1}$. The required concentration was obtained by dissolving 40 mg, 35 mg, 30 mg, 20 mg, 15 mg, and 10 mg of crude extracts in 1 ml of CH_2Cl_2 and MeOH

(1:1, v/v) separately and mixed with 19 ml portions of liquid PDA medium. Then the liquid PDA media containing crude extract was poured into Petri plates and pre-incubated overnight. A CH_2Cl_2 and MeOH mixture (1:1, v/v, 1 mL) was used as the negative control, and Nystatin was used as the positive control (40 mg of Nystatin in a 20 mL portion of liquid PDA). The next day, the inoculation was done using a small mycelia piece (around 3mm in diameter) harvested from the edge of the active *Aspergillus* spp., into the center of the PDA plate. Inoculated PDA plates were further incubated at Room Temperature for 7 days.

Results and Discussion

Screening of crude extracts for antibacterial activity

The crude extract of MeOH/CH_2Cl_2 (1:1, v/v) extraction of *K. zeylanica* (root) and crude extracts of sequential extraction of *O. herbacea* (whole plant) and *W. bicuspidata* (leaves) using Hexane, CH_2Cl_2, EtOAc and MeOH solvents were separately subjected to agar well diffusion assay to identify antibacterial activities in them. Sequential extraction is an extraction method which make it possible to obtain the maximum number of compounds present in the powdered plant material, because the chemical differences between the resulting extracts are attributable to different solubility of sample constituents in the different extraction solvents. Any natural product material is generally a complicated mixture of several compounds with different chemical and physical properties. This fact aids to find the most suitable solvent or solvents for extracting the specific class of compounds. Therefore, from sequential extraction, fats, oils, terpenes, and waxes can be extracted with hexane [16], while many caffeine compounds can be harvested with dichloromethane [17]. Also, ethyl acetate is widely used for the extraction of compounds with cytotoxic activity, and methanol is a perfect solvent for extraction of alkaloids. Thus, such compounds could be extracted with solvents of increasing polarity, depending on the chemical and physical nature of the target compounds [18]. The most suitable extraction technique for serial successive extraction is Soxhelt extraction method. But it cannot be used as thermos sensitive compounds contain in the plant material which may undergo degradation due to prolonged heating involved in that technique [19].

The inhibitory effect of the crude extracts was determined against four different bacteria strains and was quantified by measuring the diameter of the inhibition zone. Among the selected bacteria strains, Gram-positive bacteria (*S. aureus and B. cereus)* showed activity against three of the nine crude extracts tested. None of the crude extracts showed activity against the Gram-negative bacteria (*P. aeruginosa* and *E. coli)* (Table 1). *K. zeylanica* (root) extracted via MeOH/CH_2Cl_2 (1:1, v/v) exhibited significant potential antibacterial activity against both Gram-positive bacterial strains; *S. aureus* (16.9 ± 0.31mm) and *B. cereus* (16.2 ± 0.32 mm) (Figure 1). Between the four sequentially extracted fractions (Hexane, Dichloromethane, Ethyl acetate, and Methanol) of *O. herbacea*-whole plant (Figure 2) that have tested for their antibacterial activity, Ethyl acetate extract showed antibacterial activity against *S. aureus* (12.6 ± 0.31 mm) and *B. cereus* (17.7 ± 0.76 mm). None of the fractions showed any activity against *P. aeruginosa* and *E. coli.* Among the four sequentially extracted fractions (Hexane, Dichloromethane, Ethyl acetate, and Methanol) of *W. bicuspidata*-leaves (Figure 2) that have been tested for their antibacterial activity, Ethyl acetate extract showed antibacterial activity against *S. aureus* (12.3 ± 0.17 mm) only. None of the fractions showed any activity against *B. cereus*, *P. aeruginosa,* and *E. coli.*

Plant and plant part			Mean diameter of the inhibition zone (mm) ± SE: 1.0 mg/well			
			S. aureus	***B. cereus***	***P. aeruginosa***	***E. coli***
W. bicuspidata (leaves)	1	Hexane extract	-	-	-	-
	2	CH_2Cl_2 extract	-	-	-	-
	3	EtOAc extract	12.3 ± 0.17	-	-	-
	4	MeOH extract	-	-	-	-
		Positive control	36.8 ± 0.15	35.5 ± 0.34	32.5 ± 0.30	30.5 ± 0.25
O. herbacea (whole plant)	5	Hexane extract	-	-	-	-
	6	CH_2Cl_2 extract	-	-	-	-
	7	EtOAc extract	12.6 ± 0.31	17.7 ± 0.76	-	-
	8	MeOH extract	-	-	-	-
		Positive control	36.8 ± 0.15	35.5 ± 0.34	32.5 ± 0.30	30.5 ± 0.25

K. zeylanica (root)		$MeOH/CH_2Cl_2$ (1:1, v/v) extract	16.9 ± 0.31	16.2 ± 0.32	-	-
		Positive control	35.3 ± 0.33	35.0 ± 0.00	32.0 ± 0.00	30.0 ± 0.25

Table 1: Antibacterial activity of the crude plant extracts with the method of agar well diffusion assay.

[a] Crude extracts that shows zero activity against any of the four bacterial strains tested are marked by a "-" mark.

[b] Zone of inhibition for the positive control was given by gentamycin positive control.

Figure 1: Antibacterial activity of *K. zeylanica* against *S. aureus* and *B. cereus*.

Figure 2: Antibacterial activity of *O. herbacea* (whole plant) and *W. bicuspidata* (leaves) against *S. aureus.*

Screening of crude extracts for antifungal activity – Poison food technique

The crude extract acquired from $MeOH/CH_2Cl_2$ (1:1, v/v) extraction of 7 different plant parts of 5 different species were separately subjected to poison food method to identify the presence of probable antifungal activities in them. The inhibitory effect of the crude extracts was determined against three different fungal strains (*Rhizopus* spp., *Aspergillus* spp., *and Penicillium* spp.) and was calculated (Table 2) using equation 1 given under section 2.4.3.

Plant and plant part Mean diameter of growth in test plate (mm) ± SE		**Antifungal activity against *Rhizopus* spp.**		**Antifungal activity against *Aspergillus* spp.**		**Antifungal activity against *Penicillium* spp.**	
		Antifungal activity (%)	**Mean diameter of growth in test plate (mm) ± SE**	**Antifungal activity (%)**	**Mean diameter of growth in test plate (mm) ± SE**	**Antifungal activity (%)**	
A	*K. zeylanica* (stem and leaves)	82.5 ± 0.9	17.50	54.8 ± 1.0	17.34	31.2 ± 0.4	37.25
B	*O. mungos* (whole plant)	72.3 ± 0.9	26.67	14.0 ± 0.7	78.89	26.3 ± 0.3	46.98
C	*O. herbacea* (stem and leaves)	56.0 ± 0.4	44.00	41.7 ± 0.9	37.19	27.0 ± 0.4	45.64
D	*O. herbacea* (root)	100.0 ± 0.0	0.00	45.8 ± 0.6	30.90	33.2 ± 0.6	33.22
E	*W. bicuspidata* (leaves)	100.0 ± 0.0	0.00	64.8 ± 0.3	2.26	37.2 ± 0.6	25.17
F	*M. umbellata* (whole pant)	83.0 ± 1.1	17.00	54.5 ± 0.2	17.84	49.3 ± 0.6	0.67

G	*K. zeylanica* (root)	100.0 ± 0.0	0.00	65.5 ± 0.6	1.26	27.9 ± 0.6	43.79
	Negative control	100.0 ± 0.0	0.00	66.3 ± 0.9	0.00	49.7 ± 0.9	0.00
	Positive control (Nystatin)	0.0 ± 0.0	100.00	0.0 ± 0.0	100.00	0.0 ± 0.0	100.00

Table 2: Antifungal activity of the crude plant extracts with the poison food method.

Each plant extract was tested against fungal strains and was adjusted to have 1 mg ml^{-1} of final concentration before the assay was done. Among all the crude plant extracts tested, only *O. herbacea* (stem and leaves) showed significant antifungal activity (%) of 44% for *Rhizopus* spp., All other species showed less than 20% of antifungal activity except *O. mungos* (whole plant), which exhibited 27% of antifungal activity, against *Rhizopus* spp. *O. mungos* (whole plant) showed significant antifungal activity of 78.89% against *Aspergillus* spp. whilst, *O. herbacea* (stem and leaves) and *O. herbacea* (root) showed more than 30% of antifungal activity against *Aspergillus* spp. *O. mungos* (whole plant), *O. herbacea* (stem and leaves), and *K. zeylanica* (root) showed an antifungal activity between 40-47% against *Penicillium* spp. whilst *O. herbacea* (root), and *K. zeylanica* (stem and leaves) showed between 30-40% of antifungal activity against *Penicillium* spp.

Many of the crude extract tested for their antifungal activity have shown some activity against at least one fungal strain. But only 5 test results have shown significant activity (more than 40%) against any fungal strain tested. *K. zeylanica* (root), *O. mungos* (whole plant), and *O. herbacea* (stem and leaves) have shown antifungal activity between 40-50% against *Penicillium* spp. *O. herbacea* (stem and leaves) has shown antifungal activity of 44% against *Rhizopus* spp. *O. mungos* (whole plant) has shown significant antifungal activity closer to 80% against *Aspergillus* spp. (Figure 3).

Figure 3: Antifungal activity (%) of 1 mg ml-1 concentration of different crude plant extracts against *Rhizopus spp., Aspergillus spp., and Penicillium spp*. fungal strains.

Inhibitory effect of *Ophiorrhiza mungos* (whole plant) against *Aspergillus* spp.

The concentration series of *O. mungos* (whole plant) crude extract was prepared and tested against *Aspergillus* spp. to identify the inhibitory effect of *O. mungos* (whole plant) against Aspergillus spp. Even the lowest concentration (0.5 $mgml^{-1}$) tested has shown an antifungal activity of more than 50%. The highest concentration (2 $mgml^{-1}$) of *O. mungos* (whole plant) tested against *Aspergillus* spp. showed an antifungal activity of 98.5%. According to the results obtained, concentration values of more than 1.5 $mgml^{-1}$ of *O. mungos* (whole plant) crude extract show an antifungal activity of more than 90% (Figure 4).

Figure 4: Antifungal activity for the different concentrations of the plant extract of *O. mungos* (whole plant) against *Aspergillus spp*. ([B-1: 2.0 mgml-1, B-2: 1.75 mgml-1, B-3: 1.50 mgml-1, B-4: 1.0 mgml-1, B-5: 0.75 mgml-1, B-6: 0.5 mgml-1, p: positive control [Nystatin]. n: negative control)].

Conclusion

The significant antifungal activity exhibited by *O. mungos* (whole plant) against *Aspergillus* spp., and *Penicillium* spp. gives a promising indication of the availability of strong antifungal compound/s in *O. mungos* (whole plant), especially against *Aspergillus* spp. Moreover, the investigation with *O. herbacea* (stem and leaves) showed some prominent activity against all three fungal strains. This indicates the potential availability of potential antifungal compounds in *O. herbacea* (stem and leaves) against all three fungal species tested (*Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp.). Also, the results indicate that *Penicillium* spp. is the most susceptible species among the three tested fungal species in front of many crude extracts under the test. This indicates the availability of some potential antifungal compounds, targeting *Penicillium* spp., in most of the tested Rubiaceae samples that were used in the current study. On contrary, results indicated that *Rhizopus* spp. was the most resistant species among three fungal strains that have been tested. It showed a prominent susceptibility only in front of *O. herbacea* (stem and leaves) crude extract, which indicates possible availability of antifungal compound/s only in *O. herbacea* (stem and leaves) targeting *Rhizopus* spp. Ethyl acetate extracts of *W. bicuspidata* (active against only *S. aureus*) and *O. herbacea* (active against *S. aureus* and *B. cereus*) were identified as the only antibacterial active fraction extracted from large-scale sequential extraction (Hexane, CH_2Cl_2, EtOAc, and MeOH). This indicates that *O. herbacea* (whole plant) and *W. bicuspidata* (leaves) could consist of antibacterial compound/s which are moderately polar in nature considering their solubility in ethyl acetate fraction only [18]. In general, it is known that ethyl acetate is a good solvent for the extraction of cytotoxic compounds in a plant material [10]. Thus, ethyl acetate could have extracted such cytotoxic compounds, therefor as a further investigation, cytotoxic assay on ethyl acetate extract of these plant materials can be carried out as well. Also, the ethyl acetate crude extracts of *O. herbacea* (whole plant) and *W. bicuspidata* (leaves) can be subjected to the procedure of structure elucidation for the identification of their biologically active secondary metabolites that could be present in these bioactive fractions. The significant antibacterial activity exhibited by *K. zeylanica* (root) against *S. aureus* and *B. cereus* indicates the presence of antibacterial active metabolites in the root of *K. zeylanica* species. Therefore, considering these findings, it is concluded that there is an enormous possibility to isolate and identify novel sec-

ondary metabolites with prominent antifungal and antibacterial properties from the Rubiaceae species that have been selected in this study.

Acknowledgements

We extend our sincere gratitude to all members of staff in the Microbiology Laboratory in the Department of Chemistry at the University of Colombo for accommodating us in the laboratory and providing us the opportunity to carry out our work smoothly.

Conflict of Interest

There is no conflict of interest exists.

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