

## Evaluation Of *In Vitro* Antibacterial Activity of Some Sri Lankan Medicinal Plants

Athige Rajith Neloshan Silva<sup>1\*</sup>, Dissanayake Mudiyanse Ranga Kumara Dissanayake<sup>2</sup>,  
Chathuranag.Bhratheer.Ranaweera<sup>1</sup>, Ranjith Pathirana<sup>1</sup> and Wanigasekara Daya Ratnasooriya<sup>1</sup>

<sup>1</sup> Faculty of Allied Health Sciences, Department of Basic Sciences, General Sir John Kotelawala  
Defence University, Sri Lanka

<sup>2</sup> Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.

\* Email: nsrajith2005@yahoo.com

Subject: Microbiology

### Abstract

This study evaluated *in vitro* antibacterial activity and their minimum inhibitory concentration of five plants (stem of *Stereospermum suaveolens*, *Oroxylum indicum* and roots of *Clitoria ternatea*, whole plant of *Bacopa monnieri* and *Alysicarpus vaginalis*) used in traditional and folk medicine of Sri Lanka for treatment of various bacteria induced infectious diseases. This was performed using aqueous extracts against two gram positive [*Staphylococcus aureus* (ATCC 25923); *Bacillus cereus* (ATCC 11778)] and three gram negative [*Pseudomonas aeruginosa* (ATCC 9027); *Escherichia coli* (ATCC 35218); and *Salmonella typhimurium* (ATCC 13311)], clinically important bacterial pathogens, using Kirby-Bauer disk diffusion test and broth micro-dilution technique. The results showed, for the first time, that the water extract of roots of *C. ternatea* exhibits dose-related potent antibacterial activity (MIC: 128 µg/ml) against bacterial pathogen, *B.cereus*. Whilst the extracts of other four plants possessed no antibacterial activity against any of the bacterial strains tested. The antibacterial activity of *C. ternatea* roots is attributed to flavonoids, polyphenols including tannins, and saponins present in the extract. It is concluded that roots of Sri Lankan medicinal plant *C. ternatea* can function as a potent antibacterial agent against common food poisoning bacterial pathogen, *B.cereus*.

**Keywords:** *Stereospermum suaveolens*; *Oroxylum indicum*; *Indigofera tinctoria*; *Bacopa monnieri*; *Clitoria ternatea*; antibacterial activity; *Bacillus cereus*; Sri Lanka

### Introduction

Currently, there are many potent antibiotics/chemotherapeutics available for the treatment of wide range of infectious diseases mediated via bacteria.<sup>[1, 2, 3]</sup> However, unfortunately, the efficiency of the currently available antibiotics is declining substantially due to emergence of resistance resulting from their indiscriminate use.<sup>[1, 2, 3]</sup> In fact, this is a serious threat to health.<sup>[1]</sup> Further, many of the antibiotics are expensive and induce side effects, some of which are serious.<sup>[1, 3]</sup> As such, obviously, there is an urgent need for search and development of novel therapeutically active antibiotic drugs, preferably from natural/herbal sources, for the treatment of infectious diseases. In this context, it is worth noting that over 50% of all modern clinically used drugs have either natural (based on Ayurveda, traditional and folk medicine) or semisynthetic origin<sup>[4]</sup>; demand for herbal product worldwide has been increasing at an annual rate of 8%; and the estimated value of the global herbal market would

be US \$5 trillion by 2050.<sup>[5]</sup> Interestingly, today about 1500 species of medicinal plants are used in Sri Lanka, out of which around 208 plants are frequently used.<sup>[6]</sup> This study was conducted to determine the antibacterial activity and minimum inhibitory concentrations of water extracts of stem of *Stereospermum suaveolens* DC (Family: Bignoniaceae), and *Oroxylum indicum* (L.) Vent (Family: Bignoniaceae), and *Clitoria ternatea* (Family: Leguminosae) whole plant of *Alysicarpus vaginalis* (Family: Leguminosae) and *Bacopa monnieri* (L.) Pennell (Family: Plantaginaceae) against selected gram positive [*Staphylococcus aureus* (ATCC 25923); *Bacillus cereus* (ATCC 11778)] and gram negative [*Pseudomonas aeruginosa* (ATCC 9027); *Escherichia coli* (ATCC 35218); *Salmonella typhimurium* (ATCC 13311)]. In Sri Lanka ethnomedicine these plants are used for the treatment of several bacteria induced infectious diseases.<sup>[7,8,9,10]</sup>

## Material and Methods

### Collection & Authentication

Whole plant of *Bacopa monnieri* and *Alysicarpus vaginalis* and roots of *Clitoria ternatea* were collected in Beliatta (geographical coordinates: 6°1'119" North, 80°45'2700" East) situated in Hambantota district, Southern province of Sri Lanka, in October 2014. Dried pieces of stem bark of *Stereospermum suaveolens* and *Oroxylum indicum* were purchased from the drug outlet of Wickramarchchi Ayurveda Institute in Gampha district Sri Lanka in October 2014. The stem barks had been identified by the Pharmacognosist / purchasing officer of the drug outlet. Whole plant of *Bacopa monnieri* and *Alysicarpus vaginalis* and roots of *Clitoria ternatea* were identified and authenticated by Emeritus Professor (Mrs) A.S.Seneviratne, Department of Plant Sciences, University of Colombo, Sri Lanka. Voucher specimen of whole plants (BM/02/2014, AV/03/2014), stem barks (SS/04/2014, OI/05/2014) and roots (CT/05/2014) were deposited at the Department of Basic Sciences at the Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Sri Lanka.

Freeze dried plant extracts were evaluated using disc diffusion method as described by, Clinical and Laboratory Standard Institute.<sup>[11]</sup> The plant extracts were dissolved in methanol: water at a ratio of 6:4, this solvent mixture was used as the negative control. Gentamycin (10 µg/disc) was used as the positive control. The growth medium was Muller Hinton Agar and the concentrations of bacterial suspensions were compared with 0.5 McFarland standards as described by, Clinical and laboratory standard institute.<sup>[11]</sup> The antibacterial

activities were determined against *Staphylococcus aureus*(ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 35218), *Bacillus cereus* (ATCC 11778) and *Salmonella typhimurium* (ATCC 13311) at 1000 µg of plant extract/disc and 500 µg of plant extract/disc

Those were active at 500 concentration were subjected to the determination of Minimum Inhibitory Concentration (MIC value) using broth micro-dilution method with modification using Mueller Hinton Broth as the medium as described by, National Committee for Clinical Laboratory Standards.<sup>[12]</sup> The commercial antibacterial agent rifamycin was used as positive controls.

### Statistical Analysis

Data expressed as mean Inhibition zone diameter ± SEM. The results recorded were statistically analyzed by one way ANOVA using Minitab.

### Results:

As shown in Table 1, there was no antibacterial activity of root extracts of *Oroxylum indicum* and *Alysicarpus vaginalis*, stem extract of *Stereospermum suaveolens* and whole plant extract of *Bacopa monnieri*, at 1000 and 500mg/disc concentrations. On the other hand, root extract of *Clitoria ternatea* showed potent antibacterial activity only against *B. cereus strain* both at concentrations of 1000 µg/disc and 500 µg/disc with an Inhibition zone diameter of 13.0 ± 0.6 mm and 10.1 ± 0.2 mm respectively. Five hundred microgram/disc concentration of *C.ternatea*, was further subjected for the determination of Minimum Inhibitory Concentration (MIC value) against *B. cereus* and the MIC value of *C.ternatea* against *B. cereus* was found to be 128µg/ml

**Table 1: Antibacterial Screening Results of Plant Extracts (n = 3).**

Plant extract	Antibacterial activity (Inhibition zone diameter - mm)									
	<i>S. aureus</i>		<i>B. cereus</i>		<i>P. aeruginosa</i>		<i>E. coli</i>		<i>S. typhimurium</i>	
	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc
<i>Oroxylum indicum</i>	-	-	-	-	-	-	-	-	-	-
<i>Clitoria ternatea</i>	-	-	13.0 ± 0.6	10.1 ± 0.2	-	-	-	-	-	-
<i>Bacopa monnieri</i>	-	-	-	-	-	-	-	-	-	-
<i>Stereospermum suaveolens</i>	-	-	-	-	-	-	-	-	-	-
<i>Alysicarpus vaginalis</i>	-	-	-	-	-	-	-	-	-	-
Gentamycin	23.1 ± 1.0	21.7 ± 0.9	20.6 ± 0.8	22.3 ± 2.6	19.6 ± 2.1	21.4 ± 1.8	22.4 ± 1.5	24.0 ± 1.9	21.3 ± 1.9	18.5 ± 1.9

Data expressed here as mean Inhibition zone diameter ± SEM. : - indicate no antibacterial activity.

## Discussion

Peer reviewed published reports on antibacterial activity of Sri Lankan medicinal plants are limited although many research communications are available<sup>13, 14, 15</sup>. Further, many of these studies have been conducted just to find out the zone of inhibition against bacterial pathogens, but, have not investigated the MIC values. This study investigated *in vitro* antibacterial activity and minimum inhibitory concentration (MIC) of aqueous extracts (dissolved in CH<sub>3</sub>OH: H<sub>2</sub>O solvent system) of five medicinal plants commonly used by Sri Lankan traditional practitioners for various infective conditions, namely, stem of *S.suaveolens*,<sup>10</sup> roots of *C.ternatea* and *O. indicum*,<sup>9</sup> whole plant of *A. vaginalis*<sup>7</sup> whole plant of *B. monnieri*<sup>8</sup> and against two gram positive [*Staphylococcus aureus* (ATCC 25923); *Bacillus cereus* (ATCC 11778)] and three gram negative [*Pseudomonas aeruginosa* (ATCC 9027); *E. coli* (ATCC 35218); *S.typhimurium* (ATCC 13311)] clinically important bacterial pathogens using Kirby Bauer disk diffusion test and broth micro-dilution technique using Muller Hinton broth medium. These are widely used, well recognized simple validated and relatively inexpensive bacteriological techniques<sup>1</sup>. Generally, for antibacterial investigations of plant extracts, methanol is considered as the best<sup>4, 16, 17</sup> but, we have used water extracts since in traditional, folk and Ayurvedic medicine aqueous extracts (hot and cold) are used mostly as medications. Further, there are reported studies showing antibacterial activity of plants using water extracts as in the present investigation<sup>14, 15, 16</sup>.

The results unequivocally show that the water extract of roots of *C.ternatea* exhibits potent antibacterial activity (MIC: 128 µg/ml) *in vitro* against gram positive bacterial pathogen, *B. cereus* in our experimental settings. What is more is that this antibacterial action appeared to be dose-related, even though, only two concentrations were tested. However, the present study, does not pinpoint whether the antibacterial activity of the roots of *C. ternatea* is mediated via a bactericidal and/or bacteriostatic mechanisms. Nevertheless, this is a novel finding which has implications in medicine and in food industry. *B.cereus* is known to cause food poisoning with several gastrointestinal symptoms such as nausea, vomiting, epigastric pain or diarrhoea (18). In addition to food poisoning, *B.cereus* causes number of systemic and local infections in both immunologically compromised and immune competent individuals<sup>18</sup>; and *B. cereus* has a ubiquitous distribution in food (meat, rice, cereals, vegetables, fruits etc) and food products (yogurt, cheese, milk etc)<sup>18</sup>. On the other hand, the other four extracts (*S. suaveolens*, *O. indicum*,

*A.vaginalis*, *B. monnieri*) had no antibacterial activity against any of the bacterial strains tested. However, some investigators have reported *in vitro* antibacterial activity of *B. monnieri* against *E. coli*, *S.typhimurium*, *S.aureus*, and *S.cerevisiae*.<sup>16,17</sup> This discrepancy between our results and others<sup>16, 17</sup> could be due to differences in bacterial strains used, solvent extraction procedures and methods employed for detection of antibacterial activity. Alternatively, such a difference in antibacterial activity may be attributed to difference in the geographical positioning of countries from where the plant materials have originated. For example, *in vitro* antibacterial and antifungal activities have been reported in *Tribulus terrestris* from Turkey and Iran but not from Iraq<sup>19</sup>. As mentioned earlier, of the strains tested, the antibacterial activity of *C. ternatea* roots was confined only to bacterial pathogen, *B. cereus*. This indicates that *C.ternatea* aqueous root extract functions only as a narrow spectrum antibacterial. In one aspect this is a disadvantage in the treatment of acute life threatening infectious diseases where immediate antimicrobial therapy is essential<sup>1</sup>. Whilst on another aspect, it is an advantage since such an antibacterial would not disrupt the normal flora of gut that plays a vital role in excluding pathogens;<sup>1</sup> Patient who take broad spectrum antibiotics orally sometimes develop the life threatening disease called antibiotic associated colitis when normal intestinal flora are inhibited or killed. Recently, we showed, for the first time, the aqueous root extract of *C.ternatea* possess *in vitro* antirheumatic arthritic activity<sup>20</sup>; possessing of both of antibacterial and antirheumatic arthritic activity by simple plant extract is indeed a rare occurrence.

The phytochemical profile of *C. ternatea* root extract showed the presence of alkaloids, flavonoids, tannins, phenolics and saponins. Flavonoids and phenolics have been shown to mediate antibacterial activity via interactions with proteins on the bacterial cell wall and there by disrupting its functional and structural integrity<sup>19</sup>, and this is one of the key mechanisms via present day antibiotics act<sup>1, 2, 3</sup>. Such a mode of action is possible in the present study as well. Alkaloids isolated from plants are shown to possess antibacterial action which is mediated via interactions with bacterial DNA<sup>19</sup>. *C.ternatea* root extract can precipitate an antibacterial action, as evident in this study, by this mechanism too. Saponins are also shown to induce antibacterial actions through a membranolytic action<sup>19</sup>. This mode of action is also likely to be operative in the present study. Thus, *C.ternatea* root extract can provoke the antibacterial action against, *B.cereus*, as evident in this study, via a single or multiple mechanisms mentioned above.

## Conclusion

In conclusion, this study demonstrates, for the first time, potent *in vitro* antibacterial activity of an aqueous root extract of *C.ternatea* against gram positive pathogen, *B. cereus*, which causes food poisoning in man; currently there is growing interest to use herbal extracts for preservatives of food. Moreover, the result indicates a strong possibility of developing safe potent and cheap antibacterial agent from *C.ternatea* roots.

## Acknowledgements

Thanks are due to Mr H.A.K.I Dharmakeerthi and Mr H.O.T.O Perera , Mr R.Sanjaya , Ms W.A.S.Fernando for collection of the plants and Ms Bhavini Yasasri for assistance in collecting reference material.

## “Cite this Article”

ARN Silva , DM Ranga Kumara, CB Ranaweera, R Pathirana, WD Ratnasooriya  
“Evaluation Of In Vitro Antibacterial Activity of Some Sri Lankan Medicinal Plants” Int. J. of Pharm. Res. & All. Sci. 2015;4(2):54-57

## Reference

- Nester, E.W., Anderson, D.G., Roberts, C.F., Pearsall, N.N and Nester, M.T., Microbiology: A human perspective. 4<sup>th</sup> edition, McGraw Hill, New York, 2004;pp509-54
- Fauci, A.S., Braunwald, E., Kasper, D.L., Hauser, S.L., Longo, D.L., Jamason, J.L and Loscalzo, J. Harrison's Principles of Internal Medicine. Volume I, McGraw Hill, New York, 2008: pp 752-760
- Walker, B.R., Colledge, N.R., Ralston, S.H., and Penmen, I.D (Ed). Davidson's Principles and Practice of Medicine, 22<sup>nd</sup> Edition, Churchill Livingstone, London, 2014: pp 135-164
- Ruikar, A.D., Kamble, G.S., Puranik, V.G., and Deshpande, N.R, Antimicrobial screening of medicinal plant *Artemisia pallens*. Int.J.Pharmtech.Res. 2009;1: 1164-1166.
- Darmadasa, R.M., Kantinamalge, T.N.W., and Abesinghe, D.C. Ethno pharmacological survey on row materials used in traditional system of medicine in Sri Lanka: A case study, Proc. Annual symposium, Industrial Technology Institute, Colombo, Sri Lanka; pp 26
- Personal Organizer published by Sri Lanka Telecom , Colombo Sri Lanka: 2014.
- Anonymous. Osuvisithuru. Volum 1. Sri Lanka Department of Ayurveda Colombo Sri Lanka; 1994 pp 99 and 265
- Anonymous. Osuvisithuru. Volum 2. Sri Lanka Department of Ayurveda Colombo Sri Lanka; 1994 pp 305
- Anonymous. Osuvisithuru. Volum 3. Sri Lanka Department of Ayurveda Colombo Sri Lanka; 1994 pp 231
- Anonymous. Osuvisithuru. Volum 4. Sri Lanka Department of Ayurveda Colombo Sri Lanka; 1994 pp 72
- Performance standards for antimicrobial disk susceptibility test, Approved standard, Eleventh Edition, Clinical and Laboratory Standard Institute, 2006. Page 11-12
- National Committee for Clinical Laboratory Standards (2003). Performance standards for antimicrobial disc susceptibility tests, Approved Standard, document M2-A8. Wayne: National Committee for Clinical Laboratory Standards.
- Pathirane, P.T.N., Dayarathna, M.T.A and Wickramaarachchi, W.J.; The antibacterial activity of the crude extract of *Hibiscus aculeatus*. Proc. 2<sup>nd</sup> International conference on Ayurveda, Siddha and Traditional Medicine. Colombo, Sri Lanka. pp.119
- Sathiyaseelan, V., Niranjan, W., and Ravimannan, N. ;Antimicrobial and anatomical screening of the bark of *Salvadorapersica*Linn. Proc. 2<sup>nd</sup> International Conference on Ayurveda, Unani, Siddha and Traditional Medicine. Colombo, Sri Lanka. pp.124
- Dayarathna, M.T.A., Wickramaarccchi, W.J. and Jayaratne, D.L. A study of antibacterial effect of *Clerodendruminfortunatum* “Pinne” Proc. 2<sup>nd</sup>International Conference on Ayurveda, Unani, Siddha and Traditional Medicine. Colombo, Sri Lanka. pp 129
- Mathur, A., Verma, S.K., Purohit, R., Singh, S.W, Muthur, D., Prasad, G.B.K.S. and Dua, V.W.; Pharmacological investigation of *Bacapomonni* on the basis of antioxidant, antimicrobial and anti-inflammatory properties. J. Chem. Pharm.Res. 2010;2:191-198
- Liliwirianis, N., Zain, W.Z.W.M., Kassim, J., and Karim, S.A, Antimicrobial activity of plant extracts against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. E-Journal.Chem. 2011;8: S282-S284.
- Bottone, E.J. *Bacillus cereus*, a volatile human pathogen. Clin.Microbial.Rev. 2010; 23:382-398.
- Al-Bayati, F.A., Al-Mola, H.F. Antibacterial and antifungal activities of different parts of *Tribulusterrestris* L. growing in Iraq. J Zhejiang UnivSci B. 2008 Feb; 9(2): 154–159
- Ranawera, C.B.R., Pathirana, R., Chandana, A.K., Jakakody, J.R.A.C. and Rathnasooriya, W.D.; *in vitro* antirheumatic arthritic activity of aqueous root extract of *Clitoriaternatea*, Irt.Res.J.Pharmacy 2014, 5(12)