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The effect of methanolic extract of *Tamarindus indica* Linn. on the growth of clinical isolates of *Burkholderia pseudomallei*

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***Burkholderia pseudomallei* (*Pseudomonas pseudomallei*) causes melioidosis, a life-threatening infection common among paddy cultivators in Southeast Asian countries. No plant materials have been investigated for its activity against *B. pseudomallei*. Therefore, a preliminary study was carried out using disc diffusion and minimum inhibitory concentration (MIC) methods to evaluate the anti-*B. pseudomallei* activity of five Indian medicinal plants documented to have been used for several ailments in the ancient Indian scriptures. The leaf extracts of *Tamarindus indica*, *Lawsonia inermis*, and *Hibiscus rosa-sinensis*, the rhizome extracts of *Curcuma longa* and the seeds of *Vigna radiata* were prepared using methanol as solvent. The disc diffusion and MIC methods were used to assess the anti-*B. pseudomallei* activity of the plants tested. Only methanol leaf extracts of *Tamarindus indica* exhibited anti-*B. pseudomallei* activity starting from disc concentrations of 150 µg by the disc diffusion method. The other plants failed to show any zone of inhibition. MIC assay revealed that the MIC and minimum bactericidal concentration (MBC) for *B. pseudomallei* were 125 µg/ml. Our preliminary finding showed that methanolic extracts of *Tamarindus indica* has anti-*B. pseudomallei* inhibitory potentials under *in vitro* conditions. Extensive animal studies may be required before investigating the role of *Tamarindus indica* for treating melioidosis.**

Key words *Burkholderia pseudomallei* - melioidosis - *Tamarindus indica*

Melioidosis is a fatal life-threatening systemic infection caused by the gram-negative bacterium *Burkholderia pseudomallei*[1] (earlier known as *Pseudomonas pseudomallei*), that is found in soil and aquatic environments in Southeast Asia and northern Australia, and is a common cause of community-acquired infection in these regions[2]. Melioidosis has also been recognized in India[3,4]. Infection is acquired more often by direct traumatic inoculation and inhalation. Despite the availability of several prolonged combined treatment regimens for melioidosis using conventional antibiotics, relapses are common[5].

Medicinal plants are commonly used for the treatment of various ailments in India, as these are considered to have advantages over the conventionally used drugs that are expensive and known to have harmful side effects[6]. The plant parts of *Tamarindus indica*, *Curcuma longa*, *Lawsonia inermis*, *Vigna radiata*, and *Hibiscus rosa-sinensis* possess good antibacterial, anti-inflammatory and antipyretic activities[7]. Leaves of *T. indica* have already been reported to have anti-*pseudomonal* activity[6]. Therefore, a preliminary study was undertaken to assess the *in vitro* activity of five medicinal plants against clinical isolates of *B. pseudomallei*.

Four clinical isolates of *B. pseudomallei* (two each of pulmonary and blood origins) were procured from Universiti Sains Malaysia, Kubong Kerion, Kelantan, Malaysia. They were isolated from patients clinically suspected to have melioidosis. The isolates were processed in a Bio-safety Level II cabin (Airtech, India), taking essential safety precautions as *B. pseudomallei* is a hazard group 3 pathogen.

The isolates were stored in 1 per cent glycerol broth ampoules at -70ºC (Cryoscientific Ltd., Chennai) and subcultured on sheep blood agar.

The plants material, obtained from the herbarium of Indian Medical Practitioner Co-operative Society, Chennai, India (IMCOPS), was shade dried, powdered and subsequently subjected to the extraction process. The extraction was done according to Kambizi *et al*[8] with slight modifications. The plant materials tested were the leaf parts of *T. indica* (*Caesalpiniaceae*; common name Tamarind), *Lawsonia inermis* (*Lytharaceae*; common name Cypress shrub), and *Hibiscus rosa-sinensis* (*Malvaceae*; common name Shoe flower), the rhizome of *Curcuma longa* (Zingiberaceae; common name Turmeric), and seeds of *Vigna radiata* (*Fabaceae*; common name Wild black gram). To the plant powder methanol (10%) was added and incubated in a shaker for 24 h. The filtered extracts were evaporated to dryness and redissolved in methanol to obtain the required concentration. The leaf extracts were passed through a filter of 0.2 µm porosity (Sartorius, Germany) and stored at -70ºC till use.

The filter paper discs (Whatmann filter paper no.1) were sterilized by autoclaving and the extract was added onto each disc to obtain concentrations of 150, 200, 250 and 300 µg per disc. The discs thus prepared were dried before use.

Culture for drug sensitivity was carried out on Mueller-Hinton agar (Himedia, Mumbai) upon prior subculture on Mueller-Hinton broth (Himedia, Mumbai) and the inoculum used was adjusted with 0.5 McFarlard turbidity standard. Fresh cultures of *B. pseudomallei* were used for testing the plant activity. The antibacterial activity of the five different plant extracts was evaluated using the disc diffusion method and the minimum inhibitory concentration (MIC) measurements[9]. The disc inhibition zone method was evaluated by using a modification of the method described by Bauer *et al*[10]. The dried discs were placed on the test plates and results were read after overnight incubation at 37ºC. In each test, a disc containing methanol was included as a negative control. The antibacterial activity was assessed based on the measurement of diameter of the zone of inhibition (mm) formed around the discs. Simultaneously, commercial discs (Himedia, Mumbai) containing chloramphenicol (30 µg), polymyxin-B (300 U), doxycycline (30 µg) and gentamicin (10 µg) was included for each test to compare the test results.

Of the five plants tested, the methanolic extracts of *T. indica* alone showed anti-*B. pseudomallei* activity. The inhibition was observed at all concentrations tested and diameter of inhibition zones varied from 10-12 mm. Chloramphenicol (30 µg) and doxycycline (30 µg) inhibited *B. pseudomallei* giving 18 and 21 mm zones respectively whereas all isolates were resistant to polymyxin-B and gentamicin. The negative control (methanol disc) showed no inhibition of bacterial growth.

MIC measurement study was performed with tamarind leaf extract only. All procedures for inoculation, propagation, dilution, and plating were done by procedures described by Murray *et al*[11]. Sterile Mueller Hinton broth (100 µl) was added to all the wells of the microtiter plate and filter sterilized methanol extract of *T. indica* leaves at a concentration of 125 µg/ml was added to the first well, and was further serially diluted in the ratio of 1:2 until the tenth well. The eleventh well served as the control with no extract but with the inoculum and broth while the twelfth well with the inoculum and methanol. All the wells were then inoculated with freshly prepared *B. pseudomallei* cells (1 x 10^8 cfu/ml). After incubation at 37ºC for 24 h, a Klett Summerson photoelectric colorimeter (Klett MFG, USA) was used to take readings. The exact concentration that presented no growth was considered as the MIC.

The MIC value of the methanolic extracts of *T. indica* leaves against *B. pseudomallei* was 125 µg/ml. Minimum bactericidal concentration (MBC) was also determined by plating 20 µl of inoculum. The lanes with extract concentrations 300, 250 and 125 µg/ml exhibited total inhibition of *B. pseudomallei*. Hence, 125 µg/ml was also considered as the MBC for *B. pseudomallei*.

The currently available parenteral treatment regimens like ceftazidime, co-amoxyclav, chloramphenicol, doxycycline and co-trimoxazole have been documented to be potentially toxic and possess harmful side effects[5]. Co-amoxyclav therapy has well documented risks of phototoxic reactions, hypoplastic anaemia, optic neuritis and macular rashes. Chloramphenicol has predictable haemopoietic suppression and aplastic anaemia. Melioidosis responds slowly to antimicrobial therapy and relapses despite lengthy courses of treatment[5,12].

Indian medicinal plants are regularly used in various systems of medicine because of minimal side effects and cost-effectiveness[13]. Our results showed anti-*B. pseudomallei* activity of *T. indica* leaves *in vitro*. The fruits and leaves of *T. indica* have unique sour taste and possess cooling, carminative, digestive, laxative, antiscorbutic, interferon stimulatory, immunomodulatory, antiulcerogenic, hepatoprotective and antibilious properties[6,14-16].

Further attempts must be focussed on identifying the active principle from the leaves of *T. indica*, which could be an important source of new antimicrobial agent against *B. pseudomallei*. Rigorous animal studies need to be done to confirm the findings.

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References

1. Leelarasamee A, Bovornkitti S. Melioidosis: review and update. *Rev Infect Dis* 1989; *11* : 413-25.

2. Chaowagul W, White NJ, Dance DA, Wattanagoon Y, Naigowit P, Davis TM, *et al*. Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. *J Infect Dis* 1989; *159* : 890-9.

3. Jesudason MV, Anbarasu A, John TJ. Septicaemic melioidosis in a tertiary care hospital in south India. *Indian J Med Res* 2003; *117* : 119-21.

4. John TJ, Jesudason MV, Lalitha MK, Ganesh A, Mohandas V, Cherian T, *et al*. Melioidosis in India: the tip of the iceberg? *Indian J Med Res* 1996; *103* : 62-5.

5. Suputtamongkol Y, Dance DA, Chaowagul W, Wattanagoon Y, Wuthiekanun V, White NJ. Amoxycillin-clavulanic acid treatment of melioidosis. *Trans R Soc Trop Med Hyg* 1991; *85* : 672-5.

6. Khare CP, editor. In: *Encyclopaedia of Indian medicinal plant-Rational Western therapy, Ayurvedic and other traditional usage, Botany*. 2nd ed. New Delhi: Springer Verlay; 2004.

7. Sivarajan VV, Balachandran I, editors. *Ayurvedic drugs and their plant sources*. 1st ed. New Delhi: Oxford and IBH Publishers; 1998.

8. Kambizi L, Afolayan AJ. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve district, Zimbabwe. *J Ethnopharmacol* 2001; *71* : 5-9.

9. Performance standards for antimicrobial susceptibility testing. National Committee for Clinical Laboratory Standards. 9th Informational Supplement. Approved Standard M100-S9. Wayne, PA: NCCLS; 2002.

10. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; *45* : 493-6.

11. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. *Manual of clinical microbiology*. 7th ed. Washington: ASM; 1999 p. 1527-39.

12. White NJ, Dance DAB, Chaowagul W, Wattanagoon Y, Wuthiekanun V, Pitakwatchara N. Halving the mortality of severe melioidosis by ceftazidime. *Lancet* 1989; *ii* : 697-701.

13. Irfan AK, Atiya K, editors. A panorama of ethnobotany. In: *Ethnomedicine and human welfare*. Hyderabad: Ukaaz Publishers; 2004 p.18-9.

14. Ganju L, Karan D, Chanda S, Srivastava KK, Sawhney RC, Selvamurthy W. Immunomodulatory effects of agents of plant origin. *Biomed Pharmacother* 2003; *57* : 296-300.

15. Sunil G. Tamarindus indica. In: Mhaskar KS, Blatter E, Caius JF, editors. *Kirtikar and Basu's illustrated Indian medicinal plants - Their usage in Ayurveda and Unani medicines*. 3rd ed. vol. 4. Delhi: Sri Satguru Publications, Indian Book Centre; 2000 p. 1240-4.

16. Upadesh P. Medicinal plants: A to Z. In: Prajapati ND, Purohit SS, Sharma AK, Kumar T, editors. *A handbook of medicinal plants*. Jodhpur: Agrobios India; 2003 p. 502.

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