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Full Length Research Paper

In vitro interaction of combined plants: *Tinospora crispa* and *Swietenia mahagoni* against Methicillin-resistant *Staphylococcus aureus* (MRSA)

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This study has carried out to evaluate the antibacterial effect and the interaction between *Tinospora crispa* and *Swietenia mahagoni* extracts against resistant strains of Methicillin-resistant *Staphylococcus aureus* (MRSA). The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined in the setting of clinical MRSA isolates. Separated and mixture forms of *T. crispa* and *S. mahagoni* extracts were assayed to determine their antibacterial activity against eight clinical MRSA isolates. The extracts of *T. crispa* and *S. mahagoni* in separated forms were effective against MRSA strains. However, no interaction was found in the two plants combination. In conclusion, *T. crispa* and *S. mahagoni* are potential as anti MRSA agents, but the mixture of *T. crispa* and *S. mahagoni* has no synergism against MRSA strains.

Key words: Anti methicillin-resistant *Staphylococcus aureus* (MRSA), ethanolic extract, *Swietenia mahagoni, Tinospora crispa,* synergism.

INTRODUCTION

As more as attention is focused on the need to reduce the over usage of antibiotic medications for infectious diseases, even though the development of synthetic antimicrobial agents persists, drug resistance and toxicity hinder their way. There is a need for effective options for the treatment of various infection increases. For a long period of time, Herbal medications have been a valuable source of curative properties due to the presence of various complex chemical substances of different composition. Hence, the ultimate goal is to find the new medicinal plants which are pharmacologically active and free from hazardous side effects. Although the presence of S. aureus resistance to penicillin has been discovered since 1940, the extensive use of antibiotics has led to an increase in the number of resistant strains against methicillin (MRSA) (Kitai et al., 2005; Jennifer., 1999). Natural products are still the major sources of novel antibacterial agents for various diseases (Clardy et al., 2004).

Tinospora crispa, known with various vernacular names, such as “akar patawali”, or “akar seruntum”, is an indigenous plant which grows wildly in Malaysia (Noor et al., 1989). It is widely used in the traditional medicinal practice of peoples living in Malaysia, Indonesia, and Thailand to treat ailments like fever, jaundice, hyperglycemia, wounds, intestinal worms and skin infections. Moreover, *T. crispa* is also used to treat tooth and stomach aches, coughs, asthma and pleurisy, (Nik Najib et al., 1999). Scientifically speaking, *T. crispa* has been demonstrated to possess antibacterial, (Sulaiman et al., 2008) antifilarial, antimalarial, antipyretic, (Kongkathip et al., 2002) and antihyperglycaemic effects (Noor et al., 1989).

Tow triterpenes from the stem of *Tinospora crispa,* namely, cycloeucalenol and cycloeucalenone, were studied for their cardiotonic effects, compared to noradrenaline. In addition, *T. crispa* stem contains flavones O- glycosides, (apigenine), picroretoside, berberine, palmatine, picroretine, and resin, (Kongkathip et al., 2002).

Swietenia mahagoni, known as “ mahagoni,’’ belongs to the Meliaceae family, found in north and south American countries, Cuba, Jamaica, and Bangladesh. *Mahagoni* has been used in traditional medicine for the

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Table 1. List of medicinal plants used in the antimicrobial assay.

Botanical name	Family	Local name	Plant part used
Tinospora crispa	Menispermaceae	Patawali	Stem
Swietenia mahagoni	Meliaceae	Mahagoni	Seed

treatment of hypertension, diabetes, malaria, amoebiasis, coughs, chest pain and tuberculosis, and as an abortificient, antiseptic, astringent, depurative, tonic and antibacterial material against multiple - drug resistant bacterial strains, (Shahidur Rahman et al., 2009). Its extract has an ameliorative effect on diabetic mice and antimicrobial properties (Govindachari et al., 1999). Among the potential sources of new agents, medicinal plants have long been investigated. In rational drug therapy, the use of two or more drugs is often necessary and sometimes obligatory in order to achieve the desired therapeutic goal, or to treat co-existing diseases. However, the drug interaction may have different effects on the host as well as the infecting microorganism.

The potential benefits of using a combined antimicrobial therapy can be viewed as the treatment of mixed infections, the cure of severe infections in which a specific causative organism is known, the improvement of antibacterial activity, the minimization of the needed time for a long-term antimicrobial therapy, and the prevention of resistant microorganisms from emerging, (Levinson and Jawetz, 2002).

Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept that has been reported, (Betoni et al., 2006; Ali et al., 2007; Horiuchi et al., 2007). Two of the plants used in this study are medicinal plants, Tinospora crispa and *S. mahagoni*, and are considered to be antimicrobial plants. In this study, we endeavor to evaluate the antibacterial effect and the possible synergism among these plants.

MATERIALS AND METHODS

Plant material

Two plant samples are used in this study. All the plant materials are further identified in (Table 1), showing the botanical name, local name, and the plant part used.

Preparation of plant extracts

Dried and ground stems of *T. crispa,* obtained from (Ethno Resources SDN. BHD), were weighed, then homogenized in 95% ethanol, at a ratio of 1:10 and left to macerate for 3 days, at 25°C with occasional shaking and stirring. The mixture was then filtered using filter paper and the resulting liquid was concentrated under reduced pressure at 45°C to obtain a dark gummy - green extract. The concentrated extracts were then frozen and finally lyophilized with a freeze dryer, yielding the crude extract of *T. crispa* stems.

Dried and ground seeds of *S. mahagoni* were macerated in 95% ethanol, at a ratio of 1:10, for 3 days, at room temperature. The resulting extract was filtered by filter paper. The filtrate was then concentrated using rotary evaporator, at a low temperature and reduced pressure to obtain reddish- brown gummy extract. The extract was then kept in oven for two days to dry. The extract was further separated into two layers, with the oily layer above and semisolid layer at the bottom. The oily layer was poured into an empty flask and the residue was afterwards washed with hexane several times to remove any oily constituents.

Microorganisms

Eight of pure isolated MRSA strains, (Methicillin-resistant *Staphylococcus aureus*), were kindly provided by associate professor. Mohammed Yasim, Microbiology Laboratory of University of Malaysia Medical Center (UMMC).

Discs preparation

Agar disc diffusion method was applied to determine the antibacterial activity, (Miles, 1991) and according to the standards of the National Committee for Clinical technique Laboratory Standards, (NCCLS, 2003). Sterile discs (6 mm, oxoid, UK) were loaded with 4 µl (100 mg/ml) of plant mixture (1 mg/disk, 0.5 mg for each plant), dissolved in dimethyl sulfoxide (DMSO), left to dry for 6 - 10 h in sterile conditions, and then kept under 4°C. Following the same steps, sterile discs were loaded only with DMSO, only with *T. crispa* extract (0.5 mg/disk), and only with *S. mahagoni* extract (0.5 mg/disk).

Antibacterial activity

Bacterial suspensions were diluted to match the 0.5 McFarland standard scales, (approximately 1.5 x 108 CFU/ml). Muller Hinton Agar (MHA) was poured into Petri dishes to give a solid plate and inoculated with one dip of sterile cotton swab of the suspension containing 1.5 x 108 CFU/ml of bacteria. The discs treated with extracts were then placed onto Petri plates within 15 min after inoculation. Vancomycin was used as a positive control, and discs treated with DMSO were used as a negative control. The plates were then inverted and incubated at a 37°C for 16 - 18 h. The inhibition zones diameter around each of the discs was measured and recorded at the end of the incubation time. Disc diffusion test was performed in triplicate and the antibacterial activity was expressed as the means of inhibition diameters (mm), produced by the medicinal extracts.

Determination of minimum inhibition concentration

MIC was taken as the highest dilution (least concentration) of extract or drug showing no detectable growth. The minimum inhibitory concentration (MIC) values for the microorganisms were determined as the sensitivity to the extracts by checkerboard assay method, (Kumarasamy et al., 2002; Jeniffer et al., 1999). Briefly, ninety-six well-sterile plates were taken and 100 µl of plant mixture

Table 2. The Average diameter of Inhibition Zones, (IZ) for the disk diffusion tests seen on the agar plates for each type of the microorganisms tested, (Diameter of disk used = 6 mm)

Clinical MRSA*	Mean of IZ for mixture of *S. mahagoni* and *T. crispa* (mm)	Mean of IZ for *S. mahagoni* extract (mm)	Mean of IZ for *T. crispa* extract (mm)	IZ for Vancomycin antibiotic (mm)
MRSA03-22	10	5.5	5	16
MRSA03-23	19	9	9.5	15
MRSA03-25	12	6.5	6	17
MRSA04-28	12	6	7	15
MRSA04-29	10	5.5	5	15
MRSA04-30	20	10	11	17
MRSA04-31	10	6	5	15
MRSA 04-32	21	10.5	9	15

*MRSA numbers as labeled by UMMC (University of Malaya Medical Center).

of stock solution were added to row 1, and fifty microlitres of sterile normal saline were added to row 2 to 11. Two fold dilutions were performed by transferring 50 µl of extracts from row 1 to 2 using multi channel pipette. The above process was repeated up to row 12. Forty microlitres of double strength nutrient broth and 10 µl of bacterial solutions were added to all the wells, making the final concentrations of inoculums in all the wells, 5 x 106 CFU/ml. To prevent dehydration, the plates were covered with a sterile plastic cover, and then incubated at 37℃ for 16 to 18 h. The growth or non-growth was afterwards assessed (Gulluce et al., 2003; Kumar et al., 2001). The minimum bacterial count (MBC) was determined by subculturing the test dilution on a fresh drug-free, solid medium, and incubating further for 18 to 24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC, (Reube, 2008). Following the same previous steps, MIC and MBC of only *S. mahagoni,* (100 µl, 100 mg/ml) extract and MIC and MBC of only *T. crispa* (100 µl, 100 mg/ml) extract (100µl, 100 mg/ml) were determined.

RESULTS AND DISCUSSION

MRSA have become a major health risk, in terms of both nosocomial and community-acquired infections, (Eileen et al., 2007). In this work the antibacterial effect of *S. mahagoni* and *T. crispa,* and the synergism between *S. mahagoni* and *T. crispa* mixture against clinical isolated strains of (MRSA) was investigated by a disc diffusion method. The result showed different sensitivity levels for the tested strains of (MRSA), and the inhibition zone was ranged between (10 - 20) mm.

In three of the tested strains, *S. mahagoni* and *T. crispa* mixture were found to have better effect compared with vancomycin, as shown in (Table 2), while the control Dimethyl sulphoxide (DMSO) did not inhibit any of the (MRSA) isolates.

The checkerboard assay is most likely the suitable way of assessing the antibacterial potential of plant extracts. In this method, the test extract easily diffuses into the media, providing the possibility to detect small quantities of the extract antibacterial activity. Moreover, it provides the ability to distinguish between bacteriostatic and bactericidal effects and quantitative determination of minimal inhibitory concentration (MIC) (Check board assay). The MIC result was promising, as the effective mixture concentration ranged between 0.02 - 0.078 mg/ml. The sensitivity of (MRSA) strain for *S. mahagony - T. crispa* may be attributed to the 2-Hydroxy-3-O-tigloylswietenolide of *S. mahagoni* compound which, in several studies, (Eileen et al., 2007), showed antibacterial activity, in addition to N-cis-feruloyityramine, N-trans-feruloyltyramine, secoisolariciresinol, and 2, 2-diphenyl-1-pierylhydrazyl (DPPH) compound in *T crispa* extracts which were found to have antioxidant properties, (Alexandre et al.,1998).

The (MBC) was considered to be the lowest concentrated extract that did not show any bacterial growth on the agar plate after the incubation overnight, as shown in (Table 3). The (MBC) results were varied among the tested stains. In most cases, though, the (MBC) was next to the (MIC) value.

However, no synergism was observed between these two plants, as the total effect for the inhibition zone and the MIC, was not greater than the sum of the individual effects for *S. mahagoni* and *T. crispa,* in both the inhibition zone and the MIC tests.

A possible reason for the synergism's absence is the fact that the *S. mahagoni* and the *T. crispa* extracts may possibly have the same action mechanisms, or they may inhibit the same steps in the organism biosynthetic pathway, (Ofokansi, 2008).

In conclusion, our results state that the *S. mahagoni* and *T. crispa* mixture has potential antibacterial activity against (MRSA). However, no synergism interaction was found between the two investigated plants.

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Table 3. The lowest concentrations of mixture (MIC) for only *S. mahagoni*, only *T. crispa*, and *S. mahagoni* and *T. crispa* mixture were able to inhibit the visible growth of the microorganisms, and the highest dilution yielded no single bacterial colony on a solid medium after an overnight incubation (MBC).

Clinical MRSA*	*T. crispa* and *S. mahagoni* (mg/ml)		*T. crispa* (mg/ml)		*S. mahagoni* (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
MRSA03-22	0.2	0. 4	0.4	0. 78	0.2	0. 4
MRSA03-23	0. 4	0. 78	0. 4	0. 78	0. 4	0. 78
MRSA03-25	0. 78	1.56	0. 4	0. 78	0. 78	1.56
MRSA04-28	0. 78	1.56	0. 78	1.56	0. 78	1.56
MRSA04-29	0. 78	1.56	0. 78	1.56	0. 78	1.56
MRSA04-30	0. 78	1.56	0. 78	1.56	0. 78	1.56
MRSA04-31	0. 78	1.56	0. 78	1.56	0. 78	1.56
MRSA 04-32	0. 4	0. 78	0. 4	0. 78	0. 4	0. 78

REFERENCES

Ali NH, Kazmi SU, Faizi S (2007). Activity of Synergistic Combination Amoxy-Cassia Against *Salmonella,* Pak. J. Pharm. Sci., 20: 140-145.

Betoni JE, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes Junior A (2006). Synergism Between Plant Extract and Antimicrobial Drugs Used on *Staphylococcus Aureus* Diseases, Mem. Inst. Oswaldo. Cruz., 101: 387-390.

Alexandre C, Kurt H, Wahjo D, Olivier P (1998). Antioxidant and Lipophilic Constituents of *Tinospora Crispa.* Planta Med., 64(5): 393-396.

Clardy J, Walsh C (2004). Lessons from Natural Molecules. Nature, 432: 829-837.

Eileen CJS, Elizabeth MW, Neale W, Glenn WK, Simon G (2007). Antibacterial and Modulators of Bacterial Resistance from the Immature Cones of *Chamaecyparis awsoniana*. Phytochemistry, 68(2): 210-217.

Govindachari TR, Suresh G, Banumathy B, Masilamani S, Gopalakrishnan G, Krishna Kumari GN (1999) Antifungal activity of some B,D-seco limonoids from two meliaceous plants. J. Chem. Ecol., 25: 923-933.

Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A, Sahin F (2003). *In vitro* Antibacterial, Antifungal, and Antioxidant Activities of the Essential Oil and Methanol Extracts of Herbal Parts and Cullus Cultures of *Satureja Hortensis.L*. Agric. Food Chem., 51: 3958-3965.

Horiuchi K, Shiota S, Kuroda T, Hatano T, Yoshida T, Tsuchiya T (2007). Potentiation of Antimicrobial Activity of Aminoglycosides by Carnosol from *Salvia Officinalis*, Biol. Pharm. Bull., 30: 287-290.

Jennifer CS, Robert DW, John PC, Wesley EK, Carol GG, Richard JH, Ronald NJ, Michael AP (1999). Methicillin-Resistant *Staphylococcus Aureus* Outbreak in a Veterinary Teaching Hospital: Potential Human-to-Animal Transmission, J. Clin. Microbiol., 37(5): 1459–1463.

Kitai S, Akira S, Lunichi K, Eri S, Chisa N, Tatsuya U, Hiroshi K (2005). Characterization of Methicillin-Resistant *Staphylococcus Aureus* Isolated from Retail Raw Chicken Meat in Japan. Vet. Med. Sci., 67: 107–110.

Kongkathip N, Dhumma-upakorn P, Kongkathip B, Chawananoraset K, Sangchomkaeo P, Hatthakitpanichakul S (2002). Study on Cardiac Contractility of Cycloeucalenol and Cycloeucalenone Isolated from *Tinospora Crispa*. J. Ethnopharmacol., 1: 95-99.

Kumarasamy Y, Cox P, Jaspars M, Nahar L, Sarker S (2002). Screening Seeds of Scottish Plants for Antibacterial Activity. Ethnopharmacol., 83: 73-77.

Kumar S, Narain U, Tripathi S, Misra K (2001). Syntheses of Curcumin Bioconjugates and Study of their Antibacterial Activities against β-Lactamase-Producing Microorganisms. Bioconjugate Chemistry, 12: 464–469.

Levinson W, Jawetz E (2002). Medical Microbiology and Immunology: Examination and Board Review. International, ed (7th), Lange Medical.Books/McGraw-Hill, New York, pp. 73-83.

National Committee for Clinical Laboratory Standards (2003). Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard. NCCLS Document. Wayne, Pa. pp. M2-A6.

Miles DH (1991). In: Hedin PA. editor, Naturally Occurring Pest Bioregulators. ACS Symposium Series, No. 449, Am. Chem. Soc., pp. 399-406.

Nik Najib NAR, Furuta TkS, Takane K, Ali MM (1999). Antimalarial Activity of Extracts of Malaysian Medicinal Plants. Ethnopharmacol., 64: 249-254.

Noor H, Ashcroft SJ (1989). Antidiabetic Effect of *Tinospora Crispa* in Rats. Ethnopharmacology*, 27*: 149-161.

Ofokansi KC, Mbanefo AN, Ofokansi MN, Esimone CO (2008). Antibacterial Interaction of Crude Methanol Extract of *Garcinia kola* Seed with Gatifloxacin, Trop. J. Pharm. Res., 7(4): 1159-1165.

Reube KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO (2008). Phytochemical Screening and *In vitro* Antimicrobial Investigation of the Methanolic Extract of Croton Zambesicus Muell ARG. Stem Bark, Eur. J. Sci. Res., 23: 134-140.

Shahidur Rahman AKM, Azad Chowdhury AK, Husne-Ara A, Sheikh ZR, Mohammad SA, Lutfun N, Satyajit SD (2009). Antibacterial Activity of Two Limonoids from *Swietenia Mahagoni* against Multiple-Drug-Resistant (MDR) Bacterial Strains, J. Nat. Med., 63: 41-45.

Sulaiman MR, Zakaria ZA, Lihan R (2008). Antinociceptive and Anti-Inflamatory Activities of *Tinospora crispa* in Various Animal Models, Inter. J. Trop. Med., 3: 66-69.