The in vitro Antibacterial Activity of Tinospora crispa Extracts

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Abstract: The present study was carried out to evaluate the potential of aqueous (AETT), ethanol (EETT) and chloroform (CETT) extracts of Tinospora crispa as antibacterial agent against selected Gram positive (Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Streptococcus pneumoniae and Clostridium diphtheriae) and Gram negative (Shigella flexneri, Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris and Escherichia coli) bacteria using the in vitro disc diffusion methods. Twenty microliter of the extracts, prepared in the concentrations of 25, 50, 75 and 100% (stock solution) by diluting the stock solution in distilled water (D.H.O) or dimethyl sulfoxide (DMSO), were impregnated on sterilized blank discs (6 mm diameter) and tested against the respective bacteria. The AETT, at all concentrations, was effective only against S. pneumoniae and C. diphtheriae but show an activity against E. coli at the concentrations of 50% and above. At all concentrations used, the EETT was effective against S. aureus, S. pneumoniae, C. diphtheriae and S. flexneri while the CETT was effective against S. pneumoniae, C. diphtheriae and S. flexneri. Furthermore, the CETT, at the concentrations of 50% and above, was effective against E. coli. As a conclusion, T. crispa possesses potential antibacterial properties and further studies are being carried out to isolate and identify the responsible compound.

Key words: Tinospora crispa, antibacterial activity, aqueous extract, methanol extract, chloroform extract, disc diffusion method

INTRODUCTION

Many of the plants, which have been scientifically proven to possess medicinal values, were known to and used as a folklore medicine by the people of ancient cultures throughout the world as remedies to treat various ailments. According to Zaika (1975) scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century. Naturally occurring microbial inhibitors have been isolated and identified from a wide variety of plants, including garlic, onion, fruits and spices. Other than the antimicrobial activity, the extract of these plants were also reported to possess other pharmacological activities, such as analgesic, sedative, anti-inflammatory, antidiabetic and antihypertensive to name a few (Adesina, 1988).

Tinospora crispa L., locally known to the Malays as Putarwali, is a plant belonging to the family Menispermaceae. It is distributed from the southwestern part of China to Southeast Asia, including Malaysia and widely used as a traditional medicine (Noor and Ashcroft, 1998). In Malaysia, Thailand and Indonesia it is reported to be used as a bitter tonic, an antipyretic agent, an oral hypoglycemic agent, to wash wound and other skin diseases, as well as for treatment of intestinal worms' infection

T. crispa has been reported to possess antihyperglycaemic (Noor and Ashcroft, 1998) and antimalarial (Nik Najib et al., 1999) effect and effective against an infection of filarial adult worm, Brugia malayi (Zarihah, 2001). In addition, Many researchers (Yokozawa et al., 2001; Yokozawa et al., 1999; Yokozawa et al., 2000) reported on the aqueous extract of T. crispa ability to
inhibit the production of Nitric Oxide (NO), a substance that mediates various physiological events of the body when present at normal level and whose increase leads to the development of other diseases.

Based on the folklore medieval beliefs mentioned above, the objective of the current study was to screen or the presence of antibacterial activity in aqueous, methanol and chloroform extracts of T. crispum against selected Gram positive and Gram negative bacteria.

**MATERIALS AND METHODS**

T. crispum stems were purchased from a traditional medical practitioner at Kuala Krian, Kelantan, Malaysia and a voucher specimen (SK 964/04) was deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia, Malaysia.

Microorganisms tested in this study were *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Corynebacterium diphtheriae*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhi*.

Fresh stems of T. crispum were oven-dried for approximately 10 days at 40°C until there are no changes in weight according to the methods described by Somchit et al. (2003) but with slight modifications. Dried stems were then ground into fine powder under sterilized condition and stored, in a room temperature, in a dry plastic case until used. The fine powder of T. crispum stems were then soaked separately with distilled water (DH2O) (AETT), ethanol (EETT) or chloroform (CETT) in the ratio of 1:20 (w/v) for 24 h by using Soxhlet apparatus. Soaking waste residues were filtered using Whatman filter paper (No. 42) and crude extracts obtained were stored in refrigerator (-20°C) before subjected to further procedures. The resultant aqueous extract (AETT) was directly considered as a stock solution with 100% concentration/strain. On the other hand, the resultant extraction of ethanol (EETT) and chloroform (CETT) was completely evaporated (Buchi Rotavapor R-200, Germany) under pressure (Buchi Vae V-500 Pump Pressure, Germany) (Somchit et al., 2003). The obtained dried crude extracts of EETT and CETT were weighed and then dissolved in 20 mL of dimethyl sulfoxide (DMSO) and were considered as stock solutions (100% concentration/strain). All stock solutions of AETT, EETT and CETT were directly used or, dissolved using DH2O or DMSO to the concentrations of 25%, 50% or 75% before used. Twenty microliter of the respective extract were then loaded into empty sterilized blank discs (6 mm diameter, Oxoid, UK) and subjected to the disc diffusion test. In addition, commercial antibiotic discs (Flumequine; 30 μg mL⁻¹) were used for comparison.

The above-mentioned bacteria were incubated at 37°C±0.5 for 24 h after injection into nutrient broth. Mueller Hinton Agar (MHA) (Oxoid, UK), sterilized in a flask and cooled to 40-50°C, was poured (15 mL) into sterilized petri dishes (diameter of 9 cm) and allowed to harden under room temperature. This is followed by homogenous distribution of 0.1 mL bacteria cultures (10⁶ bacteria per mL) onto medium in Petri dishes. Discs loaded with extracts were then positioned on the solid agar medium by pressing slightly (Sundar, 1996). Petri dishes were placed in incubator according to their respective growth temperature and condition for 18 to 24 h. At the end of the period, inhibition zones formed was measured in mm. The study was performed in triplicate and the formation of the inhibition zones were compared with those of antibiotic discs (Flumequine; 30 μg mL⁻¹).

**RESULTS AND DISCUSSION**

As shown in the Table 1, all extracts were effective dose-dependently against the growth of *C. diphtheriae*, *S. pneumoniae* and *S. flexneri*.

Interestingly, the AETT and CETT, ranging from the concentration of 50 to 100%, were effective in inhibiting the growth of *E. coli* while those, ranging from the concentration of 75% and above, were effective against the growth of *S. aureus*.

Slight activities, for all extracts, at the concentration of 100%, were observed for treatment against *P. vulgaris*, which is also seen for treatment of AETT and EETT against *L. monocytogenes*. Unfortunately, treatment of all extracts was not effective in inhibiting the growth of *B. cereus* and *S. typhi*.

*T. crispus* L., also called putarwali or akar serutun, is widely used in Malaysia for treatment of various ailments, including skin diseases (Noor and Asheroft, 1998). Recent studies have also reported on its effectiveness as antimalarial agent (Nik Najib et al., 1999) and in inhibiting the growth of filarial adult worm (Zaridah et al., 2001). The ability of its aqueous extract to block the synthesis of NO (Yokozawa et al., 2001; Yokozawa et al., 1999; Yokozawa et al., 2000), which has been implicated as a biological messenger molecule inside and between cells with many important biochemical and physiological properties (Igarra et al., 1987; Moncada et al., 1991), might as well suggested its role in the perception of nociception (Zakaria et al., 2004).

The recent study has demonstrated the potential used of T. crispum as an antibacterial agent against infection of some of the bacteria used. Although, this
Table 1: The antibacterial activity of aqueous, methanol and chloroform extracts of *Tinospora crispa* determined by disc diffusion method

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AETT 25</th>
<th>AETT 50</th>
<th>AETT 75</th>
<th>AETT 100</th>
<th>CETT 25</th>
<th>CETT 50</th>
<th>CETT 75</th>
<th>CETT 100</th>
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<tbody>
<tr>
<td><em>C. diglomerata</em></td>
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<td>8</td>
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<td><em>S. auerus</em></td>
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<td><em>B. cereus</em></td>
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<td><em>P. vulgaris</em></td>
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<td><em>S. pneumoniae</em></td>
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<td><em>K. pneumoniae</em></td>
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<td><em>S. flexneri</em></td>
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<tr>
<td><em>E. coli</em></td>
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<td><em>S. typhi</em></td>
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<td><em>L. monocytogenes</em></td>
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</table>

IZ = Inhibition zone (mm). - No inhibition zone. Except for *B. cereus* (IZ = 10 mm), Flumequine gave inhibition zone of ≤15 mm against all bacteria tested.

The study did not show *T. crispa* as a potent antibacterial agent, when compared to the antibiotic used, it is important to bear in mind that all of the extracts used were in a crude form.

It is generally known that the crude extract might contain various types of active compounds, such as tannins and flavonoids (Diaz et al., 1988) or glycosides (Chukwurah and Ajali, 2000) or saponins (Pretorius et al., 2003), to name a few. The presence of the above-mentioned compounds abundantly in all types of plants (Pretorius et al., 2003), might help explain the observed antibacterial activity of *T. crispa* extracts.

Furthermore, although this study seems to indicate the low antibacterial activity of *T. crispa* extracts when compared to our previous studies using *Muntingia calabura* (Zakaria et al., 2005) and *Corchorus olitorius* (Zakaria et al., 2005) it is important for us to note that the method of calculating the concentration of extracts used is totally different. Different in the method of calculation the concentration, although may significantly influence the antibacterial activity of the respective plant extracts, did not seem to influence our objective to prove that the extract does possessed antibacterial activity. In addition, this is a preliminary study, carried out using the respective stock solutions that were directly diluted serially, which is concurrent with our previous study (Zakaria et al., 2004). Thus, the result may provide a basis for the isolation of compounds of biological interest from. As a conclusion, the presence study might provide some basic knowledge on the antibacterial activity of *T. crispa*.

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**REFERENCES**


