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Full Length Research Paper

Antimicrobial activity of the aqueous extract of selected Malaysian herbs

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Malaysia is rich in biodiversity encompassing a variety of herbs and shrubs with potential medicinal properties. *Tinospira crispa* (Patawali), *Anacardium occidentale* (Gajus), *Garcinia atroviridis* (Asam gelugor) and *Hibiscus cannabinus* (Kenaf) are some of the well-known local Malay herbs that have been studied for their medicinal applications. The objective of the present study was to evaluate the broad spectrum-antimicrobial effects of the aqueous extract of *T. crispa, A. occidentale, H. cannabinus* and *G. atroviridis* against *Staphylococcus aureus* ATCC 25923 (Gram positive) and *Escherichia coli* ATCC 25922 (Gram negative) bacteria using the disc diffusion method. From the results obtained, all extracts were found to exhibit a similar inhibition zone of approximately 8.67-9.67 mm against both test organisms. Furthermore, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the respective extract for both test organisms were similar; 227.27 mg/mL for *T. crispa* and *A. occidentale*, 113.64 mg/ml *for H. cannabinus*, and 56.82 mg/ml for *G. atroviridis.* In conclusion, the selected plants exhibited antimicrobial activity wherein the effectiveness (indicated by low MBC value) was in the sequence of *G. atroviridis*, *H. cannabinus*, *T. crispa* and *A. occidentale*. This findings support the traditional use of these plants in the treatment of bacterial infection.

Key words: Antimicrobial activity, *Tinospora crispa*, *Anacardium occidentale*, *Garcinia atroviridis*; *Hibiscus cannabinus*, aqueous extract, *Stapylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Antibiotic resistance has become a global concern nowadays (Westh et al., 2004) with the emergence of multidrug-resistant pathogens that has become a threat to the clinical efficacy of many existing antibiotics (Bandow et al., 2003). Thus, there is a continuous and urgent need to find or to develop new antimicrobial compounds with novel mechanisms of action for new and reemerging infectious diseases (Rojas et al., 1992). Recently, many researchers are turning their focus to folk medicine with intention of finding new leads to build better drugs against microbial infections (Benkeblia, 2004) whereby the medicinal plants were screened against various types of microbes for their potential antimicrobial activity (Wannissorn et al., 2005; Zakaria et al., 2006a, b, c, d, e, f; 2007a, b; 2010a, b; Suhaili et al., 2011). In recent years, it has been proposed that the herbal extracts may be used as natural antimicrobial agents to inhibit the growth of food borne pathogen (Lee et al., 2007) and as a source of various medicinal agents (Krishnaraju et al., 2005). Despite tremendous development in the field of modern antimicrobial drug development, medicinal plants were still considered as a good source of new antimicrobial drug candidates

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Abbreviations: MBC, Minimal bactericidal concentration; **MIC,** minimal inhibitory concentration; **ATCC,** American type culture collection; **OD,** optical density.

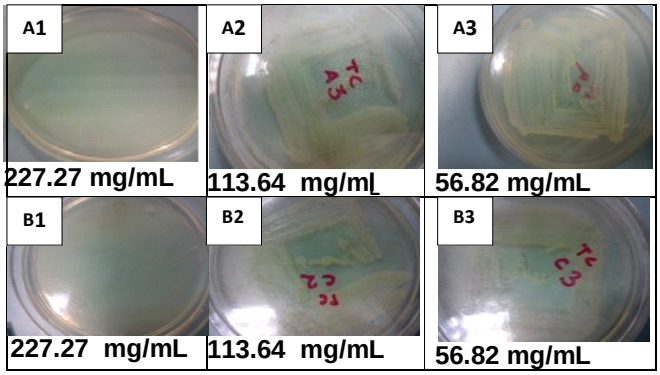

Figure 1. MBC value for *T. crispa* against (A1-A3) *S. aureus* ATCC 25923 and (B1- B3) *E. coli* ATCC 25922 was detected at 227.27 mg/ml. (Effect of dilution concentrations from 28.41 to 0.89 mg/mL was not shown).

because they are cheaper and more accessible to most of the population in the world (Igoli et al., 2002).

Four of the plants that were chosen for the present study are *Tinospora crispa*, *Anacardium occidentale*, *Hibiscus cannabinus* and *Garcinia atroviridis*. *T. crispa* is a woody and glabrous plant belonging to the family Menispermaceae, known to the Malay as “Patawali” or “Seruntum”, this climbing plant is traditionally used to treat diabetes, hypertension, stimulation of appetite and protection from mosquito bites (Zulkhairi et al., 2008). *A. occidentale*, which belongs to the family Anacardiaceae and is commonly known to the Malay as *“Gajus”*, has been used traditionally to treat many diseases such as diabetes, diarrhea, malaria and yellow fever (Akinpelu, 2001; Ayepola and Ishola, 2009). *H. cannabinus*, which is commonly called kenaf, belongs to the Malvaceae family. Various parts of *H. cannabinus* has been used to treat worm infections, pains and bruises, coughs, dysentery and bilious, blood and throat disorders, anaemia, fatigue, lassitude and biliousness with acidity (Chopra et al., 1986; Nair and Chanda, 2007). *G. atroviridis* is a medium sized fruit tree that belongs to the family Gutiferae. Known to the Malays as “Asam gelugur”, the traditional uses of this plant include in the treatment of ear ache and acne. In an attempt to establish their pharmacological properties, we studied the antibacterial activity of those plants against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion assay.

MATERIALS AND METHODS

Preparation of plant extracts

All plants’ leaves were collected from Forest Research Institute Malaysia, Kepong, Selangor, Malaysia between March and May 2010. The leaves were dried in the oven at 40°C for one week and then grinded into powder form. One hundred gram of each leaves were soaked separately in 1 L of distilled water at 60°C for 6 h and then filtered with Whatman 1 filter paper. After that, the supernatant was collected and freezed at -80°C for 24 h and freeze-dried for 48 h.

Antibiotic sensitivity test

Minimal inhibitory concentration (MIC) study

S. aureus ATCC 25923 and *E. coli* ATCC 25922 were purchased from the American Type Culture Collection (ATCC) and cultured cultured by standard methods. Three to five colonies were transferred into Mueller Hinton broth. Test tubes were incubated on a mechanical shaker at 35°C and growth phase was confirmed by monitoring growth curve in broth for 4-6 h. Turbidity was adjusted with sterile Mueller Hinton broth to match OD 0.08-0.1 turbidity standard by using spectrophotometer at 625 nm. Serial tubes containing aqueous extract of each herb from 250 mg/ml to 0.98 mg/ml were prepared using ½ dilutions. A hundred micro liter bacterial suspension was added to each serial tube by using spectrophotometer (OD standard 0.08- 0.1 at 625 nm). The serial tubes were incubated for 6-8 h.

Minimal bactericidal concentration (MBC) study

After the serial tubes were incubated for 6-8 h, each of these tubes was streaked on Mueller Hinton agar respectively by using sterile wire loop. Then, these agar plates were incubated for 24 h at 35°C.

Determination of antibacterial activity of the selected medicinal plants

The agar well diffusion technique was carried out as described by Adeniyi et al. (1996) to determine the antibacterial activity of the extracts based on the MBC obtained earlier. The tops of four to five colonies of bacteria from pure culture were picked with a sterile loop. The colonies were suspended in 5 ml of sterile physiologic saline. The inoculum was standardized to a 0.08-0.1 optical density (OD) standard by using spectrophotometer at 625 nm. The entire surface of a Mueller Hinton agar plate was inoculated using a sterile swab. Disc containing control and extracts were placed using a sterile forceps onto the agar surface and gently pressed down to ensure contact. Plates were incubated at 35°C for 6-8 h. Subsequently, the diameter of the zone of inhibition around each disk was measured.

RESULTS

The MIC and MBC values of selected plants’ extracts

The MIC and MBC of the respective extract against tested microbes were 227.27 mg/ml for *T. crispa* (Figure 1) and *A. occidentale* (Figure 2), 113.64 mg/ml for *H. cannabinus,* Figure 3), and 56.82 mg/ml for *G. atroviridis* Figure 4).

The antibacterial profile of selected medicinal plants’ extract assessed using the disc diffusion assay

The disc diffusion assay was carried out by utilizing the MBC of the respective extract using the 5 mm filter disc. In this assay, all extracts showed a narrow range of inhibition zone ranging between 8.67 to 9.67 mm (Figure

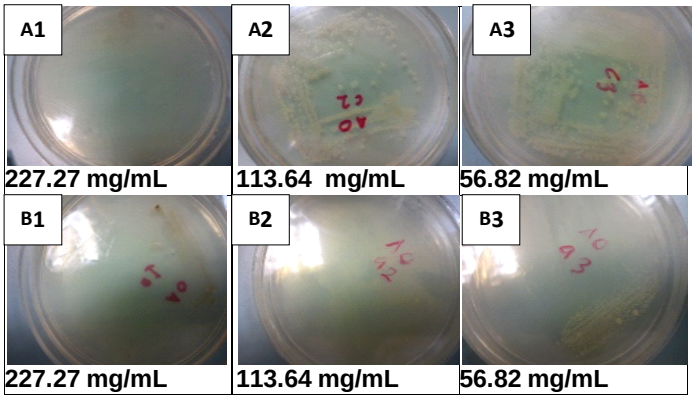

Figure 2. MBC value for *A. occidentale* against (A1-A3) *S. aureus* ATCC 25923 and (B1-B3) *E. coli* ATCC 25922 was detected at 227.27 mg/ml. (Effect of dilution concentrations from 28.41 to 0.89 mg/ml was not shown).

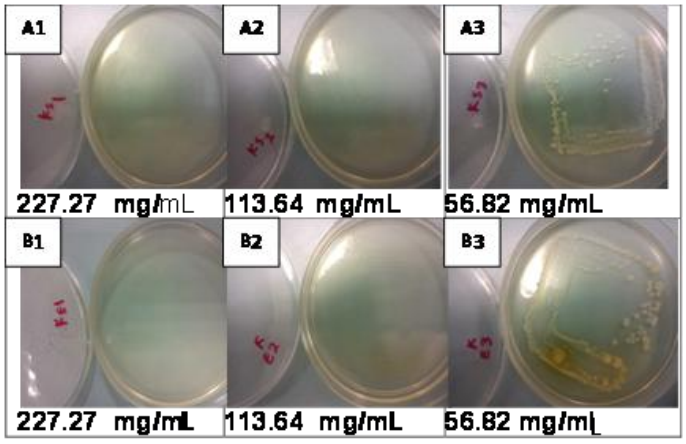

Figure 3. MBC value for *H. cannabinus* against (A1-A3) *S. aureus* ATCC 25923 and (B1-B3) *E. coli* ATCC 25922 was detected at 113.64 mg/mL. (Effect of dilution concentrations from 28.41 to 0.89 mg/mL was not shown).

5).

DISCUSSION

The result of the present study demonstrated that the aqueous extract of *T. crispa, A. occidentale, H. cannabinus* and *G. atroviridis* leaves produced zones of inhibition against tested microorganisms, *S. aureus* and *E. coli*. These observations indicate the presence of anti-bacterial activity, which confirms their medicinal potential. The ability of aqueous extract of plants to exhibit antimicrobial activity has been previously reported (Bose et al., 2007; Panda et al., 2010). Of all plants tested, *G. atroviridis* was more effective than the other plants as indicated by its lower MIC and MBC values. Overall,

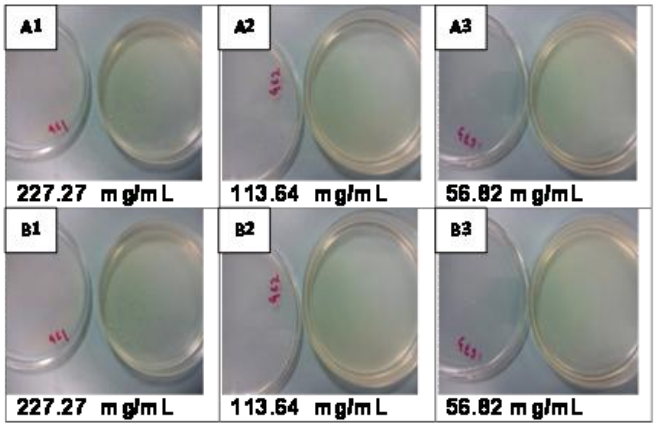

Figure 4. MBC value for *G. atroviridis* against (A1-A3) *S. aureus* ATCC 25923 and (B1-B3) *E. coli* ATCC 25922 was detected at 56.82 mg/ml. (Effect of dilution concentrations from 28.41 to 0.89 mg/ml was not shown as they gave negative results).

these findings also suggest that the responsible antimicrobial compounds were partly water-soluble. Mackeen et al. (2000) have earlier reported on the antimicrobial activity of methanol extract of various parts of *G. atroviridis* with its roots extract being the most effective one. This is followed by report of Basri et al. (2005) on the potential of ethyl acetate and ethanol extracts of *G. atroviridis* to inhibit *S. aureus* growth. However, our finding on the aqueous extract of *H. cannabinus* leaves antibacterial activity was against report made by Nair and Chanda (2007) on the failure of aqueous extract of *H. cannabinus* stem to exert anti-bacterial activity against *S. aureus*. We could not suggest whether the discrepancy in result obtained was due to different dose or type of sample used since Nair and Chanda (2007) did not clarify on the dose of aqueous extract used in their report. The present findings also demonstrated that *T. crispa* possessed antimicrobial activity, which corroborates with report by Zakaria et al. (2006a). On the other hand, Dahake et al. (2009) have earlier reported on the antimicrobial activity of the ethanol and petroleum ether extracts of *A. occidentale*, which also supported the recent findings.

Although it is not appropriate to point out to direct antibacterial mechanism that might contribute to the observed activity, several suggestions highlighted by Panda et al. (2010) could be used to explain our findings. Firstly, the water extract may involve in the disruption of lipopolysaccharide layer of gram-negative bacteria like *E. coli*, and helps to restore protein channels, which may facilitate the flow of antibacterial compounds to target sites. Secondly, the extract may negatively affect the efflux mechanism leading to a sufficient concentration of antimicrobial compounds to remain in the bacterium, thus supporting the extract inhibitory activity. Thirdly, the water extract may be inhibiting the bacterial protein synthesis

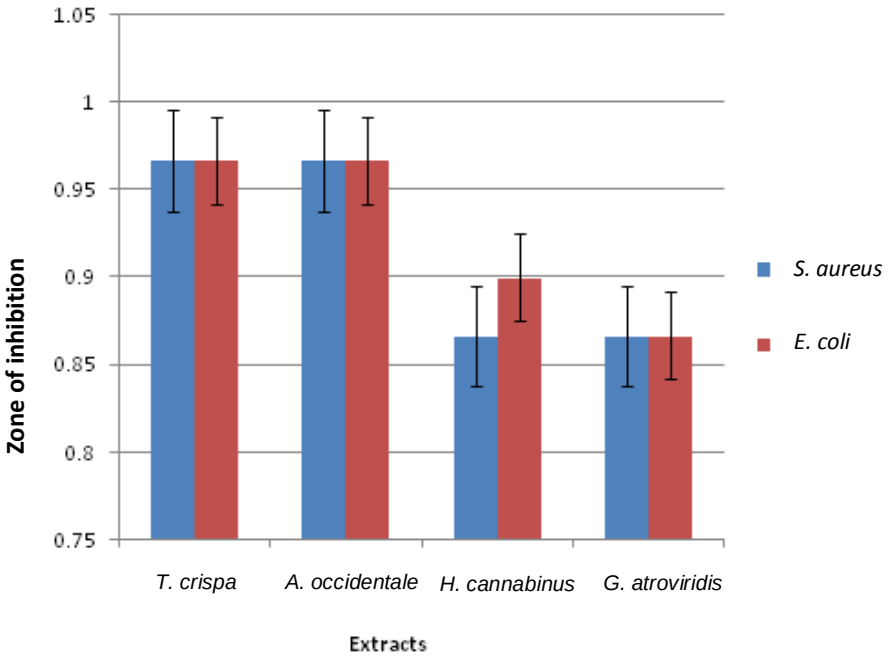

Figure 5. Antibacterial activity of selected medicinal plants against *S. aureus* and *E. coli* as indicated by the zone of growth inhibition (mm).

and fourthly, the extract may be blocking the bacterial enzymes inhibitory effects.

Phytochemical screening of *A. occidentale* leaf revealed the presence of phenolic, flavonoids, steroids and triterpenes (Fazali et al., 2011) while the aerial parts of *T. crispa* have been reported to contain various types of diterpenoids (Choudhary et al., 2010) and, triterpenes and flavonoids (Kongkathip et al., 2002). On the other hand, Silambujanaki et al. (2010) has reported on the presence of flavonoids in the methanol extract of *H. cannabinus* leaves while the leaves of *G. atroviridis* have been reported to contain phenolic compounds (Jantan et al., 2011). It is believed that these compounds contributed to the observed antimicrobial activity of the selected medicinal plants. For examples, Mbata et al. (2008) cited that flavonoids and polyphenols possess antibacterial activity.

Despite various reports on the potent *in vitro* antimicrobial activity of some of those plants, we believed that our finding only indicates mild to moderate *in vitro* antimicrobial activity since any compounds/extracts with potent *in vitro* pharmacological activity (that is, antimicrobial) should exert their activity at EC_{50}/IC_{50} value of less than or equal to 30 μg/ml (≤ 30 μg/ml) (Meyer et al., 1982; Yob et al., 2011). This discrepancy could be due to the type of extract used in the antimicrobial screening phase (Mbata et al., 2008).

Conclusion

In conclusion, the present findings further confirmed the antimicrobial potential of *G. atroviridis, H. cannabinus, T. crispa* and *A. oocindentale* leaf. Further study are required to establish the antimicrobial potential of the leaf of those plants extracted using various types of solvents and against a broad spectrum of Gram positive and Gram negative microorganisms.

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