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COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS FROM SEVERAL REGIONS OF ASIA

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ABSTRACT

This study was designed to investigate the antibacterial activities of alcohol extracts of *Phoenix dactylifera* (date palm 'type Ajwa'), *Nigella sativa* (black cumin), *Elettaria cardamomum* (cardamom), *Tinospora crispa* (Akar patawali) and *Panax ginseng* (ginseng). The plant extracts were prepared with methanol and assayed for antibacterial activity against Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and *S. aureus*. Extracts of *N. sativa*, *E. cardamomum* and *P. ginseng* produced maximum inhibition activities in the MRSA strain ATCC 33591, while *T. crispa* had the greatest activity in the strain ATCC 25923. The *P. dactylifera* extract had no effect on the tested bacteria. The growth inhibition was used to determine minimum inhibitory concentrations and minimum bactericidal concentrations. An *in vivo* experiment using the *T. crispa* ethanol extract in adult male and female Sprague Dawley rats (4 g kg^{-1} dose) showed low toxicity based on the LD_{50} value.

Keywords: Medicinal Plants, Methicillin-Resistant *Staphylococcus Aureus*, Antibacterial, Minimum Inhibitory Concentra-Tions

1. INTRODUCTION

The continent of Asia encompasses a variety of different topographies and types of soil. These differences are reflected in plant habitats as well as the composition of the plants themselves. The differences also appear in the many uses of native plants, some of which have medicinal properties. Some of the medicinal plants contain compounds that can cure infectious diseases through antibacterial, antifungal and antiviral activities (Andrade *et al*., 2011; Pereira and Gonzalez, 2004). Several studies have investigated antimicrobial agents found in medicinal plants that grow in Asia. Akhila *et al*. (2013) reported that water, ethanol, methanol and ethyl acetate extracts of *Pajanelia longifolia* had antibacterial activity against *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Lactobacillus casei* and *L. fermentum*. In addition, *Pajanelia longifolia* had significant levels of antimicrobial activity against V. *parahaemolyticus* and *B. subtilis* as well as antioxidant activity.

Methanol and ethanol extracts of *Withania somnifera* root showed antibacterial activity against *B. subtilis*, *E. coli*, *Klebsiella pnuemoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi*, compared with commercially prepared gentamicin and tetracycline antibiotics discs (Jeyanthi *et al*., 2013). The study results also indicated that the ethanol and methanol extracts of *W. somnifera* root had effective antibacterial activity against the tested bacteria compared to antibiotic discs. Moreover, the methanol extract showed more activity than the ethanol extract. It was suggested that the bioactive compounds

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were responsible for the antibacterial activity; thus, the extract offered an alternative to antibiotics that is effective, safe, ecological and economical (Jeyanthi *et al*., 2013). In another study, methanol extracts of selected plants from northwestern Punjab were screened against *S. aureus*, *E. coli* and *Pse. aeruginosa* by Gul *et al*. (2012). *Azadirachta indica* and *Mentha arvensis* extracts had high antibacterial activity against *Staphylococcus aureus*, while extracts of *Azadirachta indica*, *Cassia angustifolia*, *Phoenix dactylifera* and *Lawsonia inermis* were effective against *Candida albicans*. Extracts of *Diospyrosperegrina*, *Xylocarpus granatum*, *X. moluccensis* and *Pongamia pinnata* and the essential oils of *Citrus reticulata* cv. Murcott (honey tangerine), showed antibacterial activity against *Streptococcus equi*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *E. coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Pse. aeruginosa* (Ferhat *et al*., 2013; Wangensteen *et al*., 2013). Further, extracts and the essential oil of *Nigella sativa* have been reported to possess antimicrobial activity and *N. sativa* seeds with silver nanoparticles had antibacterial activity in the treatment of urinary tract infection (Hasan *et al*., 2013a; Ranjan *et al*., 2013).

In an investigation of the potential active compounds, *Elettaria cardamomum* was found to contain 8% volatile oils, comprising 0.2% α-phellandrene, 0.2% β-pinene, 0.3% citronellol, 0.7% γ-terpinene, 1.5% α-pinene, 1.6% myrcene, 2.7% trans-nerolidol and 2.8% sabinene; however, the volatile oil content in the seeds is dependent on storage conditions (Korikontimath *et al*., 1999). In another study, *E. cardamomum* yielded 2% to 8% essential oil, containing eucalyptol (cineole), sabinene, D-α-terpineol, acetate and borneol; 1 to 2% of the fixed oil consisted of glycerides of oleic, stearic, linolenic, palmitic, caprylic and caproic acids (Keita *et al*., 2001; Tripathi *et al*., 2002; Balaji and Chempakam, 2008). Several studies investigated the antibacterial activity of *Tinospora crispa*, *Eriobotrya japonica*, *Ficus carica* and *Phoenix dactylifera*. Zakaria *et al*. (2006) found that *T. crispa* had antibacterial activity against pathogenic bacteria. Further, methanol extracts of fruits from *E. japonica*, *F. carica* and *P. dactylifera* showed higher antibacterial activity against *E. coli*, *Pse. aeruginosa* and *S. aureus* compared with chloroform extracts, which had reduced activity in all of these bacterial species (Mahmood *et al*., 2012).

Investigations can confirm that MRSA is acquired from the community rather than hospitals; the bacteria are identified and characterized by the presence and production of the virulence factor Panton-Valentine leukocidin. Unlike nosocomial MRSA infection, which is multidrug resistant, community-acquired MRSA does not have a multidrug-resistance mechanism; it can be treated with nonmethicillin antibacterial drugs (Munckhof *et al*., 2004). However, community-acquired MRSA can adapt and develop resistance as easily as nosocomial MRSA and infections with such bacteria were reported in children and young healthy adults in France (Francis *et al*., 2005).

A study on dithiocarbamates as novel organotin compounds, the compounds have been screened for antibacterial activity against *S. aureus*, *S. typhimurium*, *P. aeruginosa* and *B. subtilis*. The results of the study showed that one of these compound is promising aganit against *S. aureus* and *S. typhi*. On other hand, cytotoxicity results on human leukemic promyelocyte HL-60 cells showed an active of two of these compounds with CD_{50} values (Awang *et al*., 2011).

In this study, we compare the antibacterial activity of plants grown in Asia. Specifically, we investigate extracts of *Phoenix dactylifera*, *Nigella sativa*, *Elettaria cardamomum*, *Tinospora crispa* and *Panax ginseng* with regard to activity against MRSA. We also assess the activity of these extracts against strains of *S. aureus* isolated from two sources: One strain from King Abdulaziz University Hospital in Jeddah and three strains from the microbiology labor-atory of the University Malaya Medical Centre.

2. MATERIALS AND METHODS

2.1. Study Bacteria

Tested bacteria included MRSA ATCC 33591 and ATCC 25923, *S. aureus* MRSA isolated from King Abdulaziz university hospital and three MRSA strains from the microbiology laboratory of the University Malaya Medical Centre (MRSA ST/0904-30, MRSA ST/0904-31 and MRSA ST/0904-32). Assay plates were prepared by inoculating 100 μL^{-1} of each sample (1×105 colony-forming units [cfu]) onto Mueller-Hinton agar (OXOID CM 337).

2.2. Study Plants and Extract Preparation

The plants were collected from different locations. *Phoenix dactylifera* was from Almadina Almo-nawara city (Saudi Arabia), *Nigella sativa* and *Elettaria cardamomum* were from Jeddah markets, *Tinospora crispa* came from Malaya and *Panax ginseng* was from China. *Nigella sativa* seeds, *P. dactylifera* and *E. cardamomum* fruits, *T. crispa* leaves and *P. ginseng* flowers were washed with distilled water several times, spread on plates and dried at 40°C. After drying, the plant materials were ground and solubilized in methanol (*P. dactylifera*, *N. sativa* and *E. cardamomum*) or ethanol (*T. crispa* and *P. ginseng*) at 50 g 100 mL^{-1}. The mixtures were kept on a 120 rpm shaker at 30°C for 24 h and were

then filtered using Whatman No. 1 filter paper. The filtered solvent was dried under reduced pressure at 40°C and the resultant deposits were used as crude extracts (Vijayakumar *et al*., 2013).

2.3. Antimicrobial Assays

The antimicrobial activity of each crude plant extract was determined in vitro against the gram-positive MRSA. The activity of each extract was measured by disc diffusion and broth dilution methods per the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2007).

For the disc diffusion method, each extract was dissolved in dimethylsulfoxide at 3 μg mL^{-1} and filtered through a 0.22 μm pore filter (Millipore, Billerica, MA). One hundred microliters of each filtered solution was placed on paper discs (1 mm diameter), which were then set on a pre-inoculated agar surface. Negative controls were prepared with each solvent. Plates were incubated at 37°C for 24 h and the inhibition zones of each disc were measured. All tests were performed in triplicate.

2.4. Minimum Inhibitory Concentration

The extracts that inhibited the growth of tested bacteria were tested to determine the Minimum Inhibitory Concentration (MIC) by using a broth-microdilution method (Wiegand *et al*., 2008). The bacteria were cultured overnight on Mueller-Hinton agar and then suspended in 1 mL^{-1} of Mueller-Hinton broth (OXOID CM 405) to give a final concentration of 5×10^5 cfu mL^{-1}. Each extract was serially diluted with Mueller-Hinton broth in a 96-well microplate and the plates were inoculated with the bacteria and incubated at 37°C for 16-20 h. After incubation, plates were evaluated for the visible presence or absence of microbial growth. MIC was determined as the lowest concentration of an extract for which there was no visible growth compared to the control (Lambert *et al*., 2001).

2.5. Minimum Bactericidal Concentration

Minimum Bactericidal Concentration (MBC) was determined for *P. dactylifera*, *N. sativa*, *E. cardamomum* and *P. ginseng* by inoculating 0.1 mL of broth from negative growth wells in the MIC assay onto sterile nutrient agar by using streak plates. The plates were incubated at 37°C for 24 h. The concentration that showed no growth of the tested organisms was considered to be the MBC; the negative control was a plate with medium only (Hernandes *et al*., 2013; Joshua and Takudzwa, 2013; Madigan *et al*., 1997).

2.6. Body Weight Analysis and Liver and Renal Function Analysis

In this *in vivo* test we focused on the *T. crispa* extract as the lees one on toxicity were studied. In order to determine a safe dosage for the plant extract, we under-took a study of the acute toxicity. Thirty-six adult male and female Sprague Dawley rats (8-10 weeks old, 180-200 g and 18 males, 18 females) were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (ethics approval number: PM 07/05/2008 MAA (a) (R)). Equal numbers of rats were assigned to one of three groups: Control (vehicle: 10% Tween-20, 5 mL kg^{-1}), or 2 or 4 g kg^{-1} of plant extract preparation. The animals were given tap water and a standard pellet diet *ad libitum* for a minimum of 5 days before the start of the treatment to allow them to accli-mate. The treatment period was 2 weeks based on OECD guidelines. The amount of the plant extract dosage given to each rat was initially calculated based on the animal's body weight (Douds, 1997). Prior to testing, all animals were fasted overnight and food was withheld for another 3 to 4 h after dosing. The acute oral toxicity study was carried out based on the OECD Guideline for Testing of OECD (2001).

Each rat's weight was recorded as suggested by OECD guidelines before the *T. crispa* extract was administered. The animals' weights were also checked on the day of termination to determine if body weight changed with treatment.

Just before termination, the animals were anesthetized with diethyl ether. Blood was drawn from the jugular vein of each rat and collected in separate BD Vacutainer® blood collection tubes with clot activator (Becton-Dickinson, Franklin Lakes, New Jersey). All samples were sent immediately to the Clinical Diagnostic Laboratory of the University Malaya Medical Centre for liver and renal function tests. Results from these tests were compared to the respective control groups for the following parameters: Total protein, albumin, globulin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (AP), creatinine and urea levels.

The LD_{50} value was estimated as being equal to the dose that would cause 50% mortality. The value of LD_{50} was also estimated as greater than the administered dose if less than 50% mortality occurred and lower if mortality exceeded 50% (Douds, 1997).

2.7. Statistical Analysis

The results were analyzed by paired-samples t-test using the IBM SPSS 20 statistical software to compare the mean values of each treatment. The results are expressed as means ± SE. Probability levels of less than 0.01 were considered highly significant.

3. RESULTS

As shown in **Table 1**, high inhibition of growth occurred in most bacteria after treatment with *N. sativa*, *E. cardamomum*, *T. crispa* and *P. ginseng* extracts. Extracts of *P. ginseng*, *E. cardamomum* and *N. sativa* were associated, respectively, with 560, 240 and 580% inhibition of MRSA ATCC 33591; these extracts yielded 260, 200 and 300% inhibition of *S. aureus*, respectively. *Tinospora crispa* extract was associated with 126%, 170, 166 and 160% inhibition for MRSA ATCC 25923, MRSA ST/0904-30, MRSA ST/0904-31 and MRSA ST/0904-32, respectively. In contrast, the extract of the *P. dactylifera* fruit did not inhibit bacterial growth. Among extracts that were associated with bacterial growth inhibition, the methanol extracts were more effective than the ethanol extracts.

The inhibition of bacterial growth was reflected in the MIC of the plant extracts (**Table 2**). In the assays with MRSA ATCC 33591, the highest MICs were 1 µg mL^{-1} for *N. sativa* and *E. cardamomum* and 2 µg mL^{-1} for P. ginseng, while MRSA ATCC 25923 assays with *T. crispa* had an MIC of 32 µg mL^{-1}. The MICs were 4, 2 and 2 µg mL^{-1} with *P. ginseng*, *N. sativa* and *E. cardamomum*, respectively, in treatments for the isolated *S. aureus*. Further, *T. crispa* treatment yielded MICs of 32, 16 and 32 µg mL^{-1} in MRSA ST/0904-30, MRSA ST/0904-31 and MRSA ST/0904-32, respectively. The MBCs of the *N. sativa*, *E. cardamomum* and *P. ginseng* extracts are shown in **Table 3**. The highest MBCs were 1 µg mL^{-1} with *N. sativa* and *E. cardamomum* and 8 µg mL^{-1} with *P. ginseng* for MRSA ATCC 33591.

Table 4 shows changes in the animals' body weight after 14 days. No significant difference was observed in any of rat groups compared to their respective control groups. The liver function parameters that were tested included serum total protein, albumin, globulin, ALT, AST and AP (**Table 5**).

Table 1. Inhibition of bacterial growth (mm) after 24 h of treatment with 100 µL of alcoholic extracts

	Panax ginseng	*Tinospora crispa*	*Elettaria cardamomum*	*Nigella sativa*	*Phoenix dactylifera*
MRSA ATCC 335910	34±0.101**	NT	17.0±0.076**	34.0±0.076**	0
MRSA ATCC 25923	NT	11.3±0.110**	NT	NT	NT
S. aureus	18±0.093**	NT	15.0±0.109**	20.0±0.076**	0
MRSA ST/0904-30	NT	13.5±0.160**	NT	NT	NT
MRSA ST/0904-31	NT	13.3±0.093**	NT	NT	NT
MRSA ST/0904-32	NT	13.0±0.174**	NT	NT	NT

NT: Not tested. **p<0.01

Table 2. MIC (µg/mL) of bacterial growth after 20 h of treatment with serial concentrations of alcoholic extracts

	Panax ginseng	*Tinospora crispa*	*Elettaria cardamomum*	*Nigella sativa*	*Phoenix dactylifera*
MRSA ATCC 33591	2	NT	1	1	-
MRSA ATCC 25923	NT	32	NT	NT	NT
S. aureus	4	NT	2	2	-
MRSA ST/0904-30	NT	32	NT	NT	NT
MRSA ST/0904-31	NT	16	NT	NT	NT
MRSA ST/0904-32	NT	32	NT	NT	NT

NT: Not tested

Table 3. MBC (µg/mL) of bacterial growth after 24 h of incubation in Mueller-Hinton agar

	Panax ginseng	*Tinospora crispa*	*Elettaria cardamomum*	*Nigella sativa*	*Phoenix dactylifera*
MRSA ATCC 33591	2	NT	1	1	-
MRSA ATCC 25923	NT	NT	NT	NT	NT
S. aureus	8	NT	4	8	-
MRSA ST/0904-30	NT	NT	NT	NT	NT
MRSA ST/0904-31	NT	NT	NT	NT	NT
MRSA ST/0904-32	NT	NT	NT	NT	NT

NT: Not tested

Table 4. Body weights of rats treated with ethanol extracts of *T. crispa*

	Mean ± SE	
Dose (g/kg)	Body weight (g) day 0	Body weight (g) day 15
4	192.33±3.06	197.16±1.66
0	194.33±2.59	197.33±2.66
0 (vehicle)	199±0.70	199.75±0.25

Table 5. Liver function analysis of rats in acute toxicity study of ethanol extract of *T. crispa*

	Mean ± SE					
Dose (g/kg)	TP (g/L)	ALP (g/L)	GLB (g/L)	ALT (IU/L)	AST (IU/L)	AP (IU/L)
4	60.67±1.80	12.00±0.51	48.66±1.47	70.50±6.86	207.50±14.63	164.66±21.11
2	61.00±1.12	12.33±0.81	48.66±0.98	69.16±3.23	209.66±7.25	166.50±23.53
0 (vehicle)	56.50±1.93	10.75±0.62	45.75±1.43	59.00±2.08	200.25±9.25	121.75±11.77

Table 6. Renal function analysis of rats in acute toxicity study of ethanol extract of *T. crispa* extracts

	(Mean ± SEM, n = 6)	
Dose (g/kg)	Creatinine (µmol/L)	Urea (mmol/L)
4	51.00±2.16	7.70±0.62
2	45.66±1.35	7.10±0.40
0 (vehicle)	50.25±2.42	7.35±0.54

Creatinine and urea levels of all groups were determined as markers of kidney function (**Table 6**). No significant changes were found in any of the liver or kidney parameters tested in comparisons of the treatment and control groups. Additionally, there were no significant differences in any parameters between males and females in any group.

The results of this study may further strengthen the recommendation for the use of the tested plants in the treatment and control of microbial infections.

4. DISSCUSION

The growth inhibition analysis in our study showed varied activity of plant extracts, the plant and the solvent type.

Our results on methanol extracts agreed with those of Joshua and Takudzwa (2013) who extracted *Mangifera indica* stem bark using four different solvents (methanol, ethyl acetate, hexane and distilled water) and testing the antibacterial efficacy of the extracts against *S. aureus*. They found that the methanol extract was the most effective. Our results also agreed with a previous study (Al-Judaibi and Al-Yousef, 2013) using methanol extracts of *Rhamnus globosa*, *Ocimum basilicum*, *Tecoma stans* and *Coleus forskohlii* against gram-positive cocci, including *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus pyogenes* and *Str. agalactiae*. The plant extracts inhibited bacterial growth and the highest inhibitory effect was achieved by treatment with *O. basilicum* in each of the five tested bacterial strains.

Screenings of the antimicrobial activity of extracts from plants, honey and black cumin oil were conducted in other studies, which showed that diethyl ether extracts were the most effective antimicrobial compounds and their activity was the most pronounced against gram-positive bacteria (Nostro *et al*., 2000; Adam, 2013).

Several studies have been performed to determine the MICs and MBCs of medicinal plant extracts, includ-ing extracts of *P. longifolia*, *Piper nigrum*, *Syzygium aromaticum*, *Pelargonium graveolens*, *Myristica fragrans*, *Origanum vulgare*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Rumex alveolatus* (leaves), *Calophyllum Rubiginosum* (stem bark) *Capsella bursa*, *Mangifera indica*, *Moringa oleifera*, *Psidium guajava*, *Murraya koenigii*, *Ficus infectoria*, *Satureja bachtiarica*, *Aristolochia indica* (leaf and flowers), *Cassia angustifolia*, *Catharanthus roseus* (leaf), *Diospyros melanoxylon*, *Dolichos biflorus*, *Gymnema sylvestre*, *Justicia procumbens*, *Dissotis perkinsiae*, *Adenocarpus mannii and Barteria fistulosa*. These studies used species of gram-negative and gram-positive bacteria and the MICs and MBCs were similar to those found in our study (Dorman and Deans, 2000; ALkhamaiseh *et al*., 2011; Kamaraj *et al*., 2012; Akhila *et al*., 2013; Choudhury *et al*., 2013; Hasan *et al*., 2013b; Sureshjani *et al*., 2013; Mohammadi-Sichani *et al*., 2013; Ndjateu *et al*., 2014).

The negative findings for *P. dactylifera* fruit extract may be due to the components being ineffective against the tested bacteria. According to Jamil *et al*. (2010), *P. dactylifera* contains inorganic elements, including K, Zn,

Ca and traces of Cr, that are required for cellular functions and may consequently support bacterial growth. The lack of activity may also be due to the solvent used and the type of bacteria. Saddiq and Bawazir (2010), for example, found that *Klebsiella pneumonia* and *Escherichia* coli growth was inhibited by treatment with *P. dactylifera* water extracts, with inhibition zones of 16.351 and 10.00 mm, respectively. Further, an acetone extract of *P. dactylifera* was shown to be a potential antiviral agent against pathogenic human viruses (Jassim and Naji, 2007).

The high antimicrobial effect of the medicinal plant extracts may be due to secondary metabolites in the plant tissues and phytochemical studies have indicated that the plants' antimicrobial activities are associated with compounds such as flavonoids, terpenes, alkaloids, tannins, hydroxyl group and phenol and essential oils such as yarrow, carvacrol, thymol, glycosides, tannins, saponins and steroids (Dorman and Deans, 2000; Choudhury *et al*., 2013; Jadha *et al*., 2013; Joshua and Takudzwa, 2013).

The findings on phytochemical compounds is in accordance with the report of Zakaria *et al*. (2006), who found that an ethanol extract of *T. crispa* was bacteriostatic against *S. aureus*. When used at higher concentrations, ethanol has a stronger extraction capacity than other solvents, which could draw out several important antibacterial substances like tannins, saponins and alka-loids (Akinyemi *et al*., 2005; Abu-Shanab *et al*., 2006; Sule and Agbabiake, 2008); the current study's findings bear out this observation as well. Aside from the high volatility of ethanol, the nature of biological active com-pounds (i.e., tannins and alkaloids) may played a role in their being chemically more susceptible to ethanol extraction (Akinyemi *et al*., 2005).

According to Sudjana *et al*. (2009), the tested organisms *Campylobacter jejuni*, *Helicobacter pylori* and *S. aureus* including MRSA were inhibited by ethanol extracts, suggesting that the plant extract acted specifically against the gram-positive cell wall due to its effectiveness against all the staphylococcal strains. The reason may be due to the extracts' hydrophobicity, which caused cell rupture as a result of the destruction of the membrane's structure (Nitta *et al*., 2002).

Extract effects on body weight were insignificant in comparisons of treatment and control groups. Liver parameters, including serum total protein, albumin, globulin, ALT, AST and AP, were also evaluated. ALT and AST are considered good markers for liver function (Hilaly *et al*., 2004; Mehta *et al*., 2009) and high serum levels of these enzymes are indicative of damage to hepatic cells (Kumar *et al*., 2009). Decreased amounts of total protein, albumin and globulin in the serum suggest chronic liver damage; most plasma proteins are synthesized in the liver and their levels can be used to evaluate the synthesizing capacity of the liver (Rasekh *et al*., 2008; Mehta *et al*., 2009). A severe histological change in the liver can be indicated by a rise in the level of serum AP and secretion of large amounts into the plasma (Sharma *et al*., 2008). A significant increase in serum levels of renal enzymes is a conventional indicator of kidney damage or nephrotoxicity (Sharma *et al*., 2008), but no such increase was observed in our present study. Overall, the extracts did not appear to have significant effects on the liver and renal function parameters in any of the treated groups or between sexes based on comparisons to control groups. However, the period of exposure to the extract was only 2 weeks, leaving open the possibility that longer exposure could still be detrimental.

5. CONCLUSION

This study showed that alcohol extracts of *Nigella sativa* (black cumin), *Elettaria cardamomum* (cardamom), *Tinospora crispa* (Akar patawali) and *Panax ginseng* (ginseng) had antibacterial effects on *S. aureus* MRSA and growth inhibition was reflected in the MICs and MBCs. Further, the ethanol extract of *T. crispa* appeared to have low toxic effects, with an LD_{50} value that exceeded 4 g kg^{-1}.

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