Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh

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\textbf{ABSTRACT}

\textbf{Context:} The resistance of bacteria to antibiotics is raising serious concern globally. Asian medicinal plants could improve the current treatment strategies for bacterial infections. The antibacterial properties of medicinal plants used by the Khyang tribe in Bangladesh have not been investigated.

\textbf{Objective:} The present study examines the antibacterial properties of 18 medicinal plants used by the Khyang tribe in day-to-day practice against human pathogenic bacteria.

\textbf{Materials and methods:} Leaves, bark, fruits, seeds, roots and rhizomes from collected plants were successively extracted with hexane, ethyl acetate and ethanol. The corresponding 54 extracts were tested against six human pathogenic bacteria by broth microdilution assay. The antibacterial mode of actions of phytoconstituents and their synergistic effect with vancomycin and cefotaxime towards MRSA was determined by time-killing assay and synergistic interaction assay, respectively.

\textbf{Results and discussion:} Hexane extract of bark of \textit{Cinnamomum cassia} (L.) J. Presl. (Lauraceae) inhibited the growth of MRSA, \textit{Enterococcus faecalis}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae} and \textit{Acinetobacter baumannii} with MIC values below 100 \textmu{g}/mL. From this plant, cinnamaldehyde evoked at 4 × MIC in 1 h an irreversible decrease of MRSA count Log10 (CFU/mL) from 6 to 0, and was synergistic with vancomycin for MRSA with fractional inhibitory concentration index of 0.3.

\textbf{Conclusions:} Our study provides evidence that the medicinal plants in Bangladesh have high potential to improve the current treatment strategies for bacterial infection.

\section*{Introduction}

The resistance of bacteria to antibiotics has increased to such extent that the World Health Organization (WHO) warns of a ‘post-antibiotic era’ (O’Neill 2014; WHO 2014). In 1998, 5\% of \textit{Escherichia coli} isolated from hospitals in the Netherlands were resistant to fluoroquinolones (Goettch et al. 2000). In 2014, five out of six WHO regions were affected with 50 or more resistance of \textit{Escherichia coli} to fluoroquinolone (WHO 2014). Carbapenem-resistant \textit{Klebsiella pneumoniae} has first been reported in Scotland in the late nineties (MacKenzie et al. 1997). In 2005, 3.3\% \textit{Klebsiella pneumoniae} isolates were resistant to carbapenem in Brooklyn hospitals (Bratu et al. 2005). In 2014, two out of six WHO regions reported 50\% or more resistance of \textit{Klebsiella pneumoniae} to carbapenem (WHO 2014). Today \textit{Acinetobacter baumannii} (Moraxellaceae) resists almost all known antibiotics (Peleg et al. 2008). The resistance of \textit{Staphylococcus aureus} (Staphylococcaceae) to methicillin emerged in 1961 (Jevons 1961). Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is now resistant to vancomycin and cefotaxime and poses a threat to human health (Fung-Tomc et al. 1988; Neu 1992; Stryjewski and Corey 2014).

In an attempt to control bacterial resistance, WHO recommends ‘to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines’ (WHO 2014). However, approval for new antibacterial agent by the FDA has been decreasing (Charles and Grayson 2004; Spellberg et al. 2004). According to Alanis (2005), the traditional antibiotic structures have been almost exhausted to the point that antibacterial research is literally crying for new chemical entities that could be found by using fresh and different research approaches. Medicinal plants in Asia have the ability to synthesize a fascinating array of low molecular weight molecules with structures completely unrelated to antibiotics. One example is the alkaloid berberine produced by \textit{Tinospora cordifolia} (Willd.) Miers ex Hook. f. & Thomson (Menispermaceae), a woody climber used in Bangladesh for the treatment of tuberculosis, cough and fever (Jahan et al. 2010). This phytoconstituent not only inhibits the growth of Gram-positive cocci \textit{Streptococcus agalactiae} (Streptococcaceae) (Peng et al. 2015), but enhances the sensitivity of \textit{Staphylococcus} strains towards antibiotics (Wojtczak et al. 2014). In addition, medicinal plants produce inhibitors of bacterial resistance (Sternitz et al. 2000). Essential oil of coriander increases the sensitivity of...
Acinetobacter baumannii to tetracycline (Duarte et al. 2012). During the last few decades, scientists from all over the world are paying much more attention to the studies of an emerging branch of science, ethnobiology, especially to tribal medicine or ethnomedicine. Since 1980s, Bangladesh with 5500 plant species and more than 100 tribal communities belonging to over a dozen linguistic groups residing in various parts of the country with diversified plant species, varied culture, and a rich traditional knowledge system, possess an ethnobotanical emporia. Due to living close to nature, the tribal communities are custodians of an unique traditional knowledge system about ambient flora, fauna, and a rich heritage of phytomedicine or ethnomedicine. Since most of these ethnic communities do not have their written scripts and language, the information about prescriptions, pharmacology, attitude towards diseases, diagnosis, etc., of the age-old tribal medicines is lying unclaimed. The people relating to advanced societies are not aware of this rich knowledge system. A country like Bangladesh has many tropical rainforest plants rich with medicinal values (Rahmatullah et al. 2010). The Khyang tribe lives in a remote area and no reports exist on their medicinal plant use. In this context, we examined the antibacterial properties of medicinal plants used by Khyang tribe in Bangladesh by broth microdilution, time-killing and synergistic interaction assay. The aims of our study were: (i) to examine antibacterial properties of 18 medicinal plants of Bangladesh towards a panel of human pathogenic bacteria, (ii) to examine the antibacterial property of at least one major phytoconstituent from the most active plant, (iii) to determine the mode of action (i.e., bacteriostatic or bactericidal) of this phytoconstituent and (iv) and to determine the effect of the phytoconstituent on the sensitivity of MRSA to vancomycin and cefotaxime. The ultimate goal of our study is to contribute to the development of safe, effective and inexpensive plant-based materials to improve the current treatment strategies for bacterial infections.

Materials and methods

Medicinal plants collection

A two-month survey for evaluation and documentation of the use of medicinal plants used for the day-to-day treatment of common diseases by traditional healers in Khyang tribe Bangladesh was performed from 5–30 April 2015 according to ethnopharmacological criteria (Cotton 1996). The survey was done on the Khyang tribe residing in villages adjoining Rowangchari bazar and Balaghata village in Bandarban district, Chittagong Hill Tracts, Bangladesh (Figure 1). Information gathered allowed the collection of 18 medicinal plants (Table 1), which were identified by Professor M. Atique Rahman, University of Chittagong. Voucher herbarium specimens with
vernacular names, collecting localities, and dates of collections were deposited at the Medicinal Plants Collection Wing, Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh. After screening of superfluous matter, the collected leaves, bark, roots, rhizomes, seeds or fruits were separated and air-dried at room temperature for 2 weeks. The dried materials were then finely pulverized by grinding using aluminium collection blender (Philips, Shanghai, China) and the powders obtained were weighted with top loading balance (Sartorius ottingen, Germany).

**Medicinal plants extraction**

The plant powders (20–60 g) were mixed at room temperature sequentially with organic solvents of increasing polarity starting with hexane (Friendemann Schmidt, Parkwood, Australia), ethyl acetate (Friendemann Schmidt, Parkwood, Australia) and 95% (v/v) ethanol (AR grade, John Kollin Corporation, Midlothian, UK) for differential extraction of non-polar, mid-polar and solvent ratio of 1:5 (w/v) for three days at room temperature. The respective liquid extracts were subsequently filtered through qualitative filter papers No. 1 (Whatman International Ltd., Maidstone, UK) using aspirator pump (EW-35031-00, 18 L/min, Rema Chemicals, Chelmsford, UK) to a concentration of 100 µg/µL. A yield for each extract was calculated.

**Table 1. Traditional therapeutic properties of 18 medicinal plants from Bangladesh.**

| Family, genus species and authority | Voucher no. | Date of collection | Locality | Common name | Part used | Traditional therapeutic use |
|------------------------------------|-------------|--------------------|----------|-------------|----------|-----------------------------|
| Apiaceae:                          |             |                    |          |             |          |                             |
| Coriandrum sativum L.              | 030         | 14 April 2015      | Balaghata| Dhone       | Seeds    | Gastrointestinal disorders  |
| Brassicaceae:                      |             |                    |          |             |          |                             |
| Brassica alba (L.) Rabenh.         | 022         | 13 April 2015      | Balaghata| Haludarisha | Seeds    | Viral infections            |
| Lepidium sativum L.                | 011         | 20 April 2015      | Rowanchari bazar | Halimdana | Seeds    | Viral infections            |
| Combreataceae:                     |             |                    |          |             |          |                             |
| Terminalia bellirica (Gaern.) Roxb.| 006         | 19 April 2015      | Rowanchari bazar | Bohera    | Fruits   | Fever, cough, dysentery, diarhoea |
| Lamiaceae:                         |             |                    |          |             |          |                             |
| Hyptis suaveolens (L.) Poit.       | 001         | 25 April 2015      | Rowanchari bazar | Tukma    | Seeds    | Gonorrhoea, fever, headache, |
| Mentha arvensis L.                 | 014         | 26 April 2015      | Rowanchari bazar | Pudinapata | Leaves  | Diarhoea, thrush            |
| Ocimum basilicum L.                | 020         | 27 April 2015      | Rowanchari bazar | Tulshibij | Leaves  | Cold, coughs, viral or bacterial infection |
| Vetiveria zizanoides (L.) Nash     | 002         | 09 April 2015      | Balaghata  | Khoskhos   | Roots    | Bacterial infection, fever |
| Lauraceae:                         |             |                    |          |             |          |                             |
| Cinnamomum cassia (L.) J. Presl.   | 035         | 18 April 2015      | Rowanchari bazar | Toz      | Bark    | Nausea, vomiting and flatulence |
| Myristicaceae:                     |             |                    |          |             |          |                             |
| Myristica fragrans Houtt.          | 063         | 19 April 2015      | Rowanchari bazar | Chakrophool | Fruits | Flatulence                  |
| Pedalceae:                         |             |                    |          |             |          |                             |
| Sesamum indicum L.                 | 021         | 26 April 2015      | Rowanchari bazar | Sadatil  | Seeds   | Watery discharge from pregnant women |
| Piperaceae:                        |             |                    |          |             |          |                             |
| Piper nigrum L.                    | 057         | 20 April 2015      | Rowanchari bazar | Sadagulmorich | Fruits | Fever, coughs, diarhoea and diabetes |
| Nigella sativa L.                  | 005         | 08 April 2015      | Balaghata  | Kalojira    | Seeds    | Pain during menstruation, diabetes |
| Zingiberaceae:                     |             |                    |          |             |          |                             |
| Curcuma caesia Roxb.               | 042         | 14 April 2015      | Balaghata  | Kala hala   | Rhizomes | Tonsillitis                 |
| Curcuma longa L.                   | 046         | 12 April 2015      | Balaghata  | Kalohalud   | Rhizomes | Skin infection              |
| Curcuma pseudomontana J. Graham    | 051         | 13 April 2015      | Balaghata  | Pahari Halud| Rhizomes | Cold                       |
| Curcuma aeruginosa Roxb.           | 052         | 13 April 2015      | Balaghata  | Kathalholud | Rhizomes | Diarhoea                    |

**Tested bacterial strains**

Stock cultures of bacteria used for this study were kindly provided by the Department of Medical Microbiology, Faculty of Medicine, University of Malaya. The following human pathogenic bacteria were used as tested organisms: Gram positive organisms MRSA (University of Malaya Hospital clinical isolate), Enterococcus faecalis (ATCC 29212) and Gram negative organisms Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 15442), Klebsiella pneumoniae (University of Malaya Hospital clinical isolate) and Acinetobacter baumannii (University of Malaya Hospital clinical isolate).

**Phytoconstituents and control antibiotics**

Cinnamaldehyde, Eugenol, gallic acid, rifampicin, vancomycin and cefotaxime were purchased from Sigma-Aldrich (St. Louis, MO, >98% purity).

**Broth microdilution assay**

Determination of minimum inhibitory concentration (MIC) was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2012). Briefly, bacterial strains were grown for 18–24 h at 37 °C. Direct suspension of the colonies were made in cationically adjusted Müeller-Hinton broth (CAMHB) and adjusted to OD625 0.08–0.1 which corresponds to 1–2 × 10⁸ CFU/mL followed by serial 10-fold dilutions to give 1 × 10⁶ CFU/mL. Bacterial suspension (50 µL) was added to 96-well round bottom microtiter plates containing an equal volume of extracts or phytoconstituents at different concentrations and the 96-well plates were incubated for 24 h at 37 °C. The MIC is defined as the lowest concentration of material tested that completely inhibits the growth of bacteria. Minimum bactericidal concentration (MBC) was determined by sub-culturing the test dilutions on to a sterile agar plate and incubated further for
18–24 h. The highest dilution that yielded 0% bacterial growth on agar plates was taken as MBC. Both MIC and MBC values were calculated as the mean of triplicate experiments. Vancomycin and rifampicin were used as positive control antibiotics.

**Time-killing assay**

Time-killing assay was conducted according to Giacometti et al. (1999). Bacteria (1 × 10⁶ CFU/mL) were incubated with cinnamaldehyde, eugenol, vancomycin or cefotaxime at 1 × MIC in Müller-Hinton broth (MHB) at 37 °C. Bacterial suspensions (10 μL) were removed at various time intervals (1, 2, 3, 4 and 5 h), serially diluted in PBS, and plated onto Müller-Hinton agar with 20–24 h at 37 °C to obtain viable colonies. Bacteria count Log₁₀ values were calculated as the mean of triplicate experiments.

**Synergistic interaction assay**

The ability of the hexane extract of bark of *Cinnamomum cassia* (L.) J. Presl. (Lauraceae), cinnamaldehyde and eugenol to increase the sensitivity of MRSA towards cefotaxime or vancomycin was measured by fractional inhibitory concentration (FIC) indices (FICIs) (Giacometti et al. 1999). Vancomycin was selected because the resistance of Gram-positive bacteria to this glycopeptide is a source of concern for clinicians (Courvalin 2006). FICI is the sum of the MIC of compound and FIC of antibiotic calculated according to the following formula (Berenbaum 1978):

\[
\text{FICI} = \text{FIC (compound)} + \text{FIC (antibiotic)}
\]

The FICI results are interpreted as such: ≤0.5 synergistic, 0.5–1 additive, 1–4 indifferent; ≥4 antagonistic (Schelz et al. 2006).

**Results and discussion**

**Medicinal plants collection**

Survey for evaluation and documentation of the use of medicinal plants used in day-to-day practice by Khyang tribe residing in villages adjoining Rowangchari bazar and Balaghata village in Bandarban district, Chittagong Hill Tracts, Bangladesh (Figure 1) conducted from 5–30 April 2015 afforded the collection of 18 plants from 11 different families (Table 1). Twelve medicinal plants out of 18 were used to treat infections and all these belong to families known to accumulate essential oils except *Terminalia bellirica* (Gaern.) Roxb. (Combretaceae) (Takhtajan 2009). The ability of plants to synthesize and accumulate essential oils is not omnipresent in plants but scattered throughout the plant kingdom in certain families (Baser and Buchbauer 2015). Kar and Jain (1971) suggested that most of the anti-infectious traditional properties of aromatic plants enlisted in indigenous system of medicine are due to their essential oil contents. Essential oils are antibacterial (Deans and Ritchie 1987).

**Table 2.** Percentage yields (w/w).

| Family, genus species | Part extracted | Hexane | Ethyl acetate | Ethanol |
|-----------------------|---------------|--------|---------------|--------|
| Apiaceae:             |               |        |               |        |
| *Coriandrum sativum*  | Seeds         | 1.8    | 1.7           | 3.3    |
| Brassicaceae:         |               |        |               |        |
| *Brassica alba*       | Seeds         | 29.6   | 6.7           | 2.0    |
| Lepidium sativum      | Seeds         | 16.4   | 2.9           | 3.6    |
| Combretaceae:         |               |        |               |        |
| *Terminalia bellirica*| Fruits        | 0.2    | 0.4           | 0.1    |
| Illiciaceae:          |               |        |               |        |
| *Illicium verum*      | Fruits        | 15.0   | 5.0           | 2.0    |
| Lamiaceae:            |               |        |               |        |
| *Hyptis suaveolens*   | Seeds         | 10.3   | 2.3           | 0.9    |
| Mentha arvensis       | Leaves        | 1.3    | 1.9           | 2.9    |
| Ocimum basilicum      | Leaves        | 9.8    | 1.7           | 0.5    |
| Vetiveria zizanioides | Roots         | 0.6    | 1.7           | 0.1    |
| Lauraceae:            |               |        |               |        |
| *Cinnamomum cassia*   | Bark          | 0.9    | 1.8           | 10.8   |
| Myristicaceae:        |               |        |               |        |
| *Myristica fragrans*  | Fruits        | 33.9   | 10.9          | 3.3    |
| Pedaliaceae:          |               |        |               |        |
| *Sesamum indicum*     | Seeds         | 34.9   | 9.4           | 0.8    |
| Piperaceae:           |               |        |               |        |
| *Piper nigrum*        | Fruits        | 2.4    | 2.6           | 2.1    |
| Ranunculaceae:        |               |        |               |        |
| *Nigella sativa*      | Seeds         | 29.4   | 5.5           | 4.3    |
| Zingiberaceae:        |               |        |               |        |
| *Curcuma caesia*      | Rhizomes      | 2.6    | 2.3           | 1.2    |
| *Curcuma longa*       | Rhizomes      | 0.1    | 1.5           | 1.2    |
| *Curcuma pseudomontana* | Rhizomes    | 0.7    | 2.6           | 2.4    |
| *Curcuma aeruginosa*  | Rhizomes      | 4.9    | 3.4           | 0.9    |
| **Average yield**     |               | 10.8   | 3.6           | 2.3    |

**Percentage yields**

The yields of extracts were calculated using the following formula:

\[
\text{Percentage yield} = \left( \frac{\text{Mass of dried extract}}{\text{Mass of dried plant part}} \right) \times 100
\]

Dried plant parts were successively extracted with hexane, ethyl acetate and ethanol to obtain lipophilic (non-polar), amphiphilic (mid-polar) and hydrophilic (polar) extracts, respectively (Harborne 1998). The average yield values ranged from 2.3 to 10.8% indicating good extraction process (Parthasarathy et al. 2008) (Table 2). Calculated averages yields for hexane, ethyl acetate and ethanol extracts were 10.8, 3.6 and 2.3%, respectively. Hexane extracts had the highest average extraction yields confirming the predominance of lipophilic natural products in the plant parts extracted (Harborne 1998).

**Broth microdilution assay**

We sought to determine the MIC of 54 extracts from the 18 plants collected by broth microdilution method (Reller et al. 2009). Results of broth microdilution assay confirmed that Gram-positive bacteria were more susceptible than Gram-negative bacteria (Table 3). Rios and Recio (2005) suggested that crude extract with MIC superior to 100 μg/mL is inactive and proposed interesting activity for MIC of 100 μg/mL and below. Fabry et al. (1998) defined active crude extracts as having MIC values below 8000 μg/mL. Kuete (2010) and Cos et al. (2006) use a stricter endpoint criteria, in which crude extracts with MIC values less than 100 μg/mL are active. Further, Kuete (2010) classifies as weakly active extracts with MIC above 625 μg/mL. Following Cos et al. (2006) and Kuete (2010), three plants had interesting activities with MIC below 100 μg/mL for at least one plant.
of the bacteria tested (Table 3). The lowest MIC towards MRSA was demonstrated by the hexane extract of Mentha arvensis (Lamiaceae) (24.3 μg/mL). According to Krishnan et al. (2010), antibacterial extracts or compounds are categorized into two classes: bacteriostatic (MBC/MIC ratio >4) and bactericidal (MBC/MIC ratio ≤4). Following this classification, hexane extract of Mentha arvensis with MBC/MIC ratio above 61.7 was bacteriostatic for MRSA; this extract was bactericidal for E. coli and bactericidal for A. baumannii. A body of experimental evidence demonstrates that it is not unusual for extracts to demonstrate equal MIC and MBC values. For instance, the ethanol extract of galls of Quercus infectoria Olivier (Fagaceae) inhibited the growth of MRSA with MIC and MBC values of 1600 μg/mL. According to Krishnan et al. (2010), bacteria with MIC and MBC values of >1500 μg/mL: high; MIC between 10 and 100 μg/mL: medium and low for MIC above 100 μg/mL. Following both these classifications, eugenol with an MIC of 11.7 μg/mL and MIC/MBC ratios of 1.0 was strongly bactericidal against E. coli, P. aeruginosa, K. pneumoniae and A. baumannii. The antibacterial potency of these phytoconstituents was quantitatively examined by broth dilution method (Table 4). Rios and Recio (2005) suggested that MIC superior to 100 μg/mL for phytoconstituent was to be avoided because it is mildly active and proposed interesting activity with MIC of 10 μg/mL and below. According to Kuete (2010), the antibacterial activity of pure compounds is classified into three categories: MIC < 10 μg/mL: high; MIC between 10 and 100 μg/mL: medium; and low for MIC above 100 μg/mL. Following both these classifications, eugenol with an MIC of 11.7 μg/mL and MIC/MBC ratios of 1.0 was strongly bactericidal against E. faecalis, E. coli and K. pneumoniae. Cinnamaldehyde was strongly bactericidal for E. faecalis. Ooi et al. (2006) tested the essential oil of Cinnamomum cassia bark and its major constituent cinnamaldehyde against a panel of bacteria and recorded activity against S. aureus and P. aeruginosa with MIC ranging from 75 to 600 μg/mL. Cinnamaldehyde, eugenol and gallic acid were moderately bactericidal for MRSA. Plant phenols, including eugenol are known for their membrane-disturbing activities (Sikkema et al. 1995). This mechanism of activity could at least account for the antibacterial properties of gallic acid (Smith et al. 2005; Borges et al. 2013). The different spectrum of activity between cinnamaldehyde and eugenol could at least be explained by the fact that.

| Family, genus species | Part extracted | Extracts | MRSA | E.f | E.c | P.a | K.p | A.b |
|-----------------------|---------------|---------|------|-----|-----|-----|-----|-----|
| Apiceae:              |               |         |      |     |     |     |     |     |
| Coriandrum sativum seeds | E             | 375     | 187.5| 187.5| 187.5| 187.5| 375 |
| Brassicaceae:          |               |         |      |     |     |     |     |     |
| Brassica alba          | Seeds         | H       | 375  | 187.5| 750  | >1500| 375 | 375 |
| Combrutecae:           |               |         |      |     |     |     |     |     |
| Terminalia bellirica   | Fruits        | EA      | 187.5| >1500| 187.5| >1500| 187.5| 375 |
| Illiciaceae:           |               |         |      |     |     |     |     |     |
| Illicum verum         | Fruits        | H       | 375  | 750  | >1000| 187.5| 375 | 375 |
| Ocimum basilicium      |               |         |      |     |     |     |     |     |
| Vetiveria zizanioides  | Roots         | H       | 187.5| 750  | 375  | >1500| 375 | 1500 |
| Lauraceae:             |               |         |      |     |     |     |     |     |
| Cinnamomum cassia      | Bark          | H       | 46.8/187.5 | 46.8/375 | 46.8/93.8 | 93.8/93.8 | 46.8/375 | 11.7/46.8 |
| Apiaceae:              |               |         |      |     |     |     |     |     |
| Coriandrum sativum seeds | E             | 375     | 187.5| 375  | >1500| 187.5| 375 | 375 |
| Myristicaceae:         |               |         |      |     |     |     |     |     |
| Myristica fragans      | Fruits        | H       | 187.5| 750  | 375  | >1500| 375 | 375 |
| Pedalaceae:            |               |         |      |     |     |     |     |     |
| Sesamum indicum        | Seeds         | H       | 375  | 375  | 375  | >1500| 375 | 375 |
| Piperaceae:            |               |         |      |     |     |     |     |     |
| Piper nigrum           | Fruits        | H       | 187.5| 750  | 375  | >1500| 375 | 1500 |
| Zingiberaceae:         |               |         |      |     |     |     |     |     |
| Curcuma longa          | Rhizomes      | H       | 750  | >1500| 375  | >1500| 375 | 375 |

Positive control antibiotics:

- Vancomycin: 1500/1500 μg/mL. Values are given as mean of triplicate.
- Rifampicin: n.t. Values are given as mean of triplicate.
polar antibacterial agents can pass the outer membrane through porin channels, whereas the outer membrane serves as a penetration barrier towards macromolecules (like vancomycin) and to non-polar compounds, and it is for this reason that Gram-negative bacteria are relatively resistant to non-polar molecules (Nikaido and Vaara 1985).

**Time-killing assay**

Cinnamaldehyde and eugenol were tested against MRSA for time-killing assay as this bacterium represents the greatest current medical need (Ling et al. 2015). The result of time-killing assay is presented in Table 5. A perusal of this table shows that cinnamaldehyde (4 × MIC) and eugenol at both 2 and 4 × MIC evoked at 1 h a fall of Log_{10} (CFU/mL) bacteria count from 6 to 0. This effect was permanent confirming bactericidal activity. Gill and Holley (2006) made the demonstration that cinnamaldehyde at high concentration was bactericidal on *E. coli* via inhibition of membrane-bound ATPase activity. This small molecular weight molecule being lipophilic may penetrate and destabilize the cytoplasmic membrane of MRSA leading to nutrients and energy depletion (Sikkema et al. 1995). In previous study, cinnamaldehyde was inhibitory for the growth of the enteric bacteria but exhibited neither outer membrane-disintegrating activity nor depletion of intracellular ATP (Helander et al. 1998). In addition, aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain the observed activity (Moleyar and Narasimham 1986). Such electronegative compounds may interfere in biological processes involving electron transfer and reaction with vital nitrogen components, e.g., proteins and nucleic acids, and therefore inhibit the growth of the microorganisms. Cinnamaldehyde may also bind to amino acids in enzymes via its carbonyl group (Wendakoon and Sakaguchi 1993).

**Synergistic interaction assay**

Cinnamaldehyde has been reported to be synergistic with ampicillin, penicillin, tetracycline or novobiocin against *E. coli* (Palaniappan and Holley 2010). Eugenol is a constituent of *Cinnamomum cassia* bark. In this context, we sought to determine the synergy effects of the hexane extract of *Cinnamomum cassia*, cinnamaldehyde or eugenol, and antibiotics against methicillin-resistant *Staphylococcus aureus*.

| Phytoconstituent | Plant of origin | MRSA a/C2 | E. f. b/C2 | E. c. b/C2 | P. a. b/C2 | K. p. b/C2 | A. b. b/C2 |
|------------------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Cinnamaldehyde   | *Cinnamomum cassia* | 750/1125  | 11.7/11.7 | 23.4/23.4 | 1500/1500 | 750/1125  | 1500/1500 |
| Eugenol          | *Cinnamomum cassia* | 750/1125  | 11.7/11.7 | 11.7/11.7 | 23.4/23.4 | 11.7/11.7 | 1500/1500 |
| Gallic acid      | *Terminalia bella* | 750/1500  | 1500/1500 | 750/750   | 750/750   | 750/1500  | 750/1500  |

Table 4. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of three phytoconstituents by broth microdilution assay.

| Extract | FICI<sup>a</sup> | Effect<sup>b</sup> |
|---------|-----------------|-------------------|
| Cefotaxime | >4.2 | Antagonistic |
| Vancomycin | >4.2 | Antagonistic |
| Cinnamaldehyde | | |
| Cefotaxime | 2.2 | Indifferent |
| Vancomycin | 0.3 | Synergistic |
| Eugenol | | |
| Cefotaxime | >4.2 | Antagonistic |
| Vancomycin | >4.2 | Antagonistic |

Table 5. Time-killing assay of eugenol and cinnamaldehyde against methicillin-resistant *Staphylococcus aureus*.

Table 6. Fractional inhibitory concentration index (FICI) of different combination of hexane extract of *Cinnamomum cassia*, cinnamaldehyde or eugenol, and antibiotics against methicillin-resistant *Staphylococcus aureus*.

<sup>a</sup>Values are given as mean of triplicate. n.t.: not tested.

<sup>b</sup>MRSA: methicillin-resistant *Staphylococcus aureus*; E.f: *Enterococcus faecalis*; E.c: *Escherichia coli*; P.a: *Pseudomonas aeruginosa*; K.p: *Klebsiella pneumoniae*; A.b: *Acinetobacter baumannii*.

<sup>c</sup>Hexane extract of bark of *Cinnamomum cassia*.
antibacterial activity. From this plant, cinnamaldehyde is a resistant-modifying agent that decreases the resistance of MRSA to vancomycin. Our study provides evidence that the medicinal plants in Bangladesh have high potential for the development of plant-based material to improve the current treatment strategies for bacterial infections.

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Disclosure statement

We have no conflict of interest to declare.

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