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ORIGINAL ARTICLE

Phytochemical screening and antimicrobial potentials of *Borreria* sps (Rubiaceae)

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Abstract Successive hexane, acetone, ethanol and methanolic whole plant extracts of the *Borreria* sps were investigated for phytochemical screening and assessed for antimicrobial activity. Phytochemical analysis of *Borreria* sps extracts revealed the presence of phenolics, flavonoids and tannins. Among them, *Borreria laevicaulis* hexane extracts were found to be most effective showing the largest zone of inhibition against *Staphylococcus aureus* (22.15 mm) and *Candida albicans* (25.65 mm). Further studies indicated that the minimum inhibitory concentration of *B. laevicaulis* hexane extracts was found to be 62.5 μg/ml against *S. aureus* and 250 μg/ml against *C. albicans* and the zone of inhibitions was significantly higher than nystatin (positive control). Together, we provide new insights of the *B. laevicaulis* as a potential candidate for antimicrobial drug discovery using in vitro studies that might be useful to treat human infectious diseases and antibiotic resistant pathogens.

1. Introduction

Infectious diseases are fatal and life threatening throughout the world. The amplification of diseases is largely due to indiscriminate use of antibiotics (Avila et al., 2008). Recent studies have extensively addressed the dramatic increase of microbial resistance to antibiotics (Triyana, 2009; Kumar et al., 2006) and methods to treat them. Antimicrobial resistance evolved through mutation and genetic exchange systems which render the elimination of diseases becomes ineffective. Hence, there is an urge to continuously search for alternative sources including natural products. Traditional medicinal systems like Ayurvedic, Chinese Medicine, and Unani developed from plant resources have been used to treat various diseases. The isolation of bioactive compounds such as tannins, terpenoids, alkaloids, flavonoids etc. for potential drug discovery has been extensively reported (Choudhury et al., 2012; Taylor, 2013).

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Rubiaceae is well known for its medicinal values, used in the treatment of malaria, diarrhea, digestive problems, skin diseases, fever, hemorrhage, urinary and respiratory infections, headache, inflammation of eyes and gums (Conserva and Ferreira, 2012). Most species of Rubiaceae are normally grow as weeds in Malaysia due to their strong habitat adaptation ability. *Borreria exilis* (L.O. Williams) C.D. Adams is an annual herb distributed throughout the tropical countries, America and Africa (iNaturalist.org, 2013), used for treating headache (Conserva and Ferreira, 2012).

Borreria laevicaulis (Miq.) Ridl. is an annual or perennial herb naturalized along tropical Asia, Africa, Mauritius and East India. Traditionally, it is used as a poultice for headache; wounds healer; plant sap to treat eczema, worms and ringworm (Pravat and Prithwiraj, 2012). Leaf juice is applied for ringworm and eczema while the plant sap is used to treat the wound or lesion (Ebana et al., 1991). *Borreria latifolia* (Aubl.) K. Schum. is an annual herb that grows as a dominant weed on waste areas or agricultural fields and is normally distributed in India, Southeast Asia and Malaysia. It possesses antimicrobial properties against *Bacillus cereus*, *Bacillus megaterium* and *Pseudomonas aeruginosa* (Choudhury et al., 2012).

Borreria remotifolia DC is an annual herb widely distributed in tropical Asia, Africa, Australia and the Pacific Islands (FOC, 2013). Different plant parts have been used as antidotes to cure venomous stings and bites; roots as a medicinal are used to cure tetanus (Allabi et al., 2011). *Richardia brasiliensis* Gomes is an annual or perennial herb mainly distributed in the Southeastern United States, Asia, Midwest, South and Southeast of Brazil. It is traditionally used as an expectorant, antiemetic, diaphoretic, anti-inflammatory and in several treatments for hemorrhoids, coughs, bronchitis and headache (Hall et al., 2012). Phytochemical study revealed the presence of coumarin, flavonoids, steroids, terpenoids, alkaloids and resins in aerial parts of the plant (Morais et al., 2013). In the current study, we tested the phytochemical contents and antimicrobial activity of five *Borreria* sps against different pathogenic bacteria and fungal strains.

2. Materials and methods

2.1. Collection of plant materials

B. exilis, *B. latifolia*, *B. laevicaulis*, *B. remotifolia* and *R. brasiliensis* were collected from various locations around Kelantan, Malaysia. All the plant samples were identified by a botanist, from the University of Malaysia, Kelantan.

2.2. Preparation of plant extracts

The fresh plant samples (whole plant parts) collected were washed individually under running tap water and dried in an oven at 40 °C for 3 days. The dried plant materials were ground into powder using an electrical blender. About 10 g of dry powdered plant material from each plant was extracted by soxhlation using various solvents like methanol, ethanol, acetone and hexane. Extracts were then concentrated using a rotary evaporator and the concentrated residual extracts were stored at 4 °C in a dry airtight container until further use.

2.3. Microbial culture, inoculum preparation

Pathogenic bacterial and fungal strains were tested for the antimicrobial activity using *Borreria* sps plant extracts. Tested strains included gram positive bacteria such as *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (clinical isolates); Gram negative bacteria such as *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Klebsiella pneumoniae* (clinical isolates), and fungi such as *Candida albicans* (clinical isolates), *Aspergillus niger* (clinical isolates). All American Type Culture Collection was obtained from the

Table 1 Qualitative analysis of phytochemicals from whole plant extracts of the *Borreria* sps.

		Phenolic	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins
		$FeCl_3$	Mayer	NaOH	Braymer	Salkowki	Foam test
Borreria exilis	Methanol	+	–	+	+	+	–
	Ethanol	+	–	+	+	+	–
	Acetone	+	+	+	+	–	–
	Hexane	–	+	–	–	–	–
Borreria laevicaulis	Methanol	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+
	Acetone	+	+	+	+	–	+
	Hexane	+	+	+	+	–	–
Borreria latifolia	Methanol	+	–	+	+	–	–
	Ethanol	+	–	+	+	–	–
	Acetone	+	–	+	+	–	–
	Hexane	+	–	+	+	–	–
Borreria remotifolia	Methanol	+	–	+	+	–	+
	Ethanol	+	–	+	+	–	–
	Acetone	+	–	+	+	–	–
	Hexane	–	+	+	–	–	–
Richardia brasiliensis	Methanol	+	+	+	+	–	+
	Ethanol	+	–	+	+	–	–
	Acetone	+	–	+	+	–	–
	Hexane	–	–	–	–	–	–

Note: +, indicates presence of phytochemicals; –, indicates absence of phytochemical.