

RESEARCH ARTICLE

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Thai ethnomedicinal plants as resistant modifying agents for combating *Acinetobacter baumannii* infections

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Abstracts

Background: *Acinetobacter baumannii* is well-recognized as an important nosocomial pathogen, however, due to their intrinsic resistance to several antibiotics, treatment options are limited. Synergistic effects between antibiotics and medicinal plants, particularly their active components, have intensively been studied as alternative approaches.

Methods: Fifty-one ethanol extracts obtained from 44 different selected medicinal plant species were tested for resistance modifying agents (RMAs) of novobiocin against *A. baumannii* using growth inhibition assay.

Results: At 250 µg/ml, *Holarrhena antidysenterica*, *Punica granatum*, *Quisqualis indica*, *Terminalia bellirica*, *Terminalia chebula*, and *Terminalia* sp. that possessed low intrinsic antibacterial activity significantly enhanced the activity of novobiocin at 1 µg/ml (1/8xminimum inhibitory concentration) against this pathogen. *Holarrhena antidysenterica* at 7.8 µg/ml demonstrated remarkable resistant modifying ability against *A. baumannii* in combination with novobiocin. The phytochemical study revealed that constituents of this medicinal plant contain alkaloids, condensed tannins, and triterpenoids.

Conclusion: The use of *Holarrhena antidysenterica* in combination with novobiocin provides an effective alternative treatment for multidrug resistant *A. baumannii* infections.

Background

An underestimated nosocomial pathogen, *Acinetobacter baumannii*, is now widely acknowledged as a common bacterium in hospital irrigation and intravenous solutions. It possesses inherent multidrug-resistance (MDR) and the ability to rapidly colonize and infect patients. Moreover, the emergence of acquired MDR by *A. baumannii* to conventional antibiotics presents a serious therapeutic problem in the treatment of the infections [1,2]. Several investigations suggested that synergy effects of plant secondary metabolites and conventional antibiotics could be an alternative way to increase the bacterial susceptibility [3-6].

Plants, particularly ethnomedicinal plants are important sources of natural products. They are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids and have been well-established to possess antimicrobial properties [7]. Many plants have

been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistant modifying agent (RMA) [4].

Novobiocin, a Gyr B inhibitor, is an effective aminocoumarin drug for the treatment of Gram-positive bacterial infections. However, its low level of activity against Gram-negative pathogens causes a major limitation [8]. Although, several investigations observed synergy and mechanisms of action between natural products and synthetic drugs in effectively combating Gram positive bacterial infections [5], there are a few RMA effective for use with *A. baumannii* [9,10]. Therefore, the aim of this study was to further explore the resistant modifying activity of a wide range of medicinal plants according to their ethnobotanical basis in combination with novobiocin against *A. baumannii*.

Methods

Bacterial strain and culture condition

Acinetobacter baumannii ATCC 19606 was employed in this study as a model reference strain. The strain was susceptible to ciprofloxacin, colistin, imipenem, and

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Table 1 Intrinsic antibacterial activity and resistant modifying ability of crude extract (250 µg/ml) in combination with novobiocin (1/8xMIC) against *Acinetobacter baumannii* ATCC 19606

	Botanical names	Family name	Part used	%Growth inhibition ^a ± SD ^b		Interpretation ^c
				PE	PE + NOV	
1	<i>Aegle marmelos</i> (L.) Corr. Serr.	Rutaceae	Fruit	22.10 ± 0.68	27.10 ± 1.38	No synergy
2	<i>Ardisia colorata</i> Roxb.	Primulaceae	Fruit	30.17 ± 2.56	39.00 ± 6.09	Synergy
3	<i>Asclepias curassavica</i> L.	Asclepiadaceae	Wood	40.81 ± 0.28	43.59 ± 1.78	No synergy
4	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Whole	19.09 ± 1.06	23.93 ± 2.87	No synergy
5	<i>Cinnamomum bejolghota</i> (Buch.-Ham.) Sweet	Lauraceae	Wood	58.84 ± 1.37	59.92 ± 1.78	No synergy
6	<i>Cinnamomum porrectum</i> (Roxb.) Kosterm.	Lauraceae	Bark	55.62 ± 4.98	62.44 ± 2.91	No Synergy
			Wood	29.72 ± 6.54	26.06 ± 5.21	No synergy
7	<i>Curcuma longa</i> L.	Zingiberaceae	Rhizome	56.88 ± 2.14	63.31 ± 4.87	No synergy
8	<i>Curcuma zedoaria</i> (Christm.) Roscoe	Zingiberaceae	Rhizome	86.91 ± 2.64	88.78 ± 2.08	No synergy
9	<i>Derris scandens</i> Benth.	Leguminosea	Stem	77.73 ± 0.48	79.59 ± 2.62	No synergy
10	<i>Dracaena loureiri</i> Gagnep.	Agavaceae	Wood	49.01 ± 2.37	47.31 ± 3.84	No synergy
11	<i>Dryopteris symmactica</i> (Willd.) Kuntze	Dryopteridaceae	Stem	30.08 ± 0.99	29.49 ± 3.19	No synergy
12	<i>Eleutherine americana</i> (Aubl.) Merr. ex K.	Iridaceae	Bulb	17.59 ± 0.41	26.66 ± 5.32	Synergy
13	<i>Euphorbia thymifolia</i> L.	Euphorbiaceae	Whole plant	17.87 ± 1.89	22.26 ± 3.12	No synergy
14	<i>Garcinia mangostana</i> L.	Clusiaceae	Pericarp	53.64 ± 0.90	73.99 ± 0.88	Synergy
15	<i>Gymnopetalum cochinchinensis</i> (Lour.) Kurz	Cucurbitaceae	Fruit	93.25 ± 3.65	90.48 ± 3.37	No synergy
16	<i>Holarrhena antidysenterica</i> (L.) Wall. ex A. DC.	Apocynaceae	Bark	26.17 ± 0.59	32.45 ± 4.39	No synergy
17	<i>Impatiens balsamina</i> L.	Balsaminaceae	Stem	65.88 ± 0.11	94.04 ± 0.59*	Synergy
18	<i>Manilkara achras</i> (Mill.) Fosb.	Sapotaceae	Fruit	9.77 ± 0.30	12.40 ± 1.56	No synergy
19	<i>Millingtonia hortensis</i> L.f.	Bignoniaceae	Flower	56.59 ± 1.02	63.06 ± 2.97	No synergy
20	<i>Mitragyna speciosa</i> Korth.	Rubiaceae	Leaf	28.97 ± 4.30	54.08 ± 0.83	Synergy
21	<i>Momordica charantia</i> L.	Cucurbitaceae	Vine	43.33 ± 2.40	66.15 ± 0.26	Synergy
22	<i>Morinda citrifolia</i> L.	Rubiaceae	Fruit	22.26 ± 0.85	25.79 ± 3.10	No synergy
23	<i>Murdannia loriformis</i> (Hassk.) R. Rao & Kammathy	Commelinaceae	Whole plant	16.96 ± 0.63	25.86 ± 1.22	Synergy
24	<i>Oroxylum indicum</i> (L.) Vent.	Bignoniaceae	Leaf	16.42 ± 1.51	22.04 ± 1.67	No synergy
25	<i>Peltophorum pterocarpum</i> (DC.) Backer ex K. Heyne	Fabaceae	Flower	67.18 ± 1.59	71.30 ± 5.28	No synergy
			Bark	42.80 ± 0.43	47.83 ± 4.49	No synergy
26	<i>Piper betle</i> L.	Piperaceae	Leaf	78.26 ± 0.60	88.75 ± 6.10	Synergy
			Leaf	42.72 ± 0.13	39.92 ± 3.43	No synergy
27	<i>Piper nigrum</i> L.	Piperaceae	Fruit	38.07 ± 1.96	42.24 ± 2.60	No synergy
			Seed	29.07 ± 0.75	31.47 ± 3.27	No synergy
28	<i>Piper retrofractum</i> Vahl	Piperaceae	Fruit	44.02 ± 1.08	49.80 ± 4.19	No synergy
29	<i>Piper sarmentosum</i> Roxb	Piperaceae	Leaf	20.70 ± 0.88	25.02 ± 0.62	No synergy
30	<i>Pluchea indica</i> (L.) Less.	Asteraceae	Leaf	26.64 ± 0.97	53.59 ± 3.60*	Synergy
31	<i>Psidium guajava</i> L.	Myrtaceae	Leaf	71.24 ± 2.00	81.19 ± 1.50*	Synergy
32	<i>Punica granatum</i> L.	Puniceaceae	Pericarp	72.58 ± 1.20	99.29 ± 0.63*	Synergy
33	<i>Quercus infectoria</i> G.Olivier	Fagaceae	Gall	89.09 ± 0.15	88.77 ± 1.00	No synergy

Table 1 Intrinsic antibacterial activity and resistant modifying ability of crude extract (250 µg/ml) in combination with novobiocin (1/8xMIC) against *Acinetobacter baumannii* ATCC 19606 (Continued)

34	<i>Quisqualis indica</i> L.	Combretaceae	Flower	79.22 ± 0.28	94.63 ± 2.62*	Synergy
35	<i>Rhizophora mucronata</i> Lam.	Rhizophoraceae	Fruit	44.64 ± 0.59	53.35 ± 2.56	Synergy
			Bark	42.68 ± 8.20	53.03 ± 4.95	Synergy
36	<i>Rhodomyrtus tomentosa</i> (Aiton) Hassk.	Myrtaceae	Stem	77.01 ± 1.28	81.81 ± 4.01	No synergy
37	<i>Sandoricum indicum</i> Cav.	Meliaceae	Root	65.24 ± 1.32	66.94 ± 2.13	No synergy
38	<i>Tamarindus indica</i> L.	Fabaceae	Leaf	19.76 ± 1.55	25.03 ± 3.45	No synergy
39	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Fruit	74.79 ± 0.53	95.68 ± 1.14*	Synergy
40	<i>Terminalia chebula</i> (Gaertn.) Retz.	Combretaceae	Fruit	61.25 ± 0.42	94.33 ± 1.95*	Synergy
41	<i>Terminalia</i> sp.	Combretaceae	Fruit	79.53 ± 0.24	95.92 ± 1.10*	Synergy
42	<i>Theobroma cacao</i> L.	Sterculiaceae	Pericarp	17.35 ± 0.74	22.81 ± 0.68	No synergy
			Seed	19.25 ± 1.08	29.61 ± 4.13	Synergy
43	<i>Vitex trifolia</i> L.	Verbenaceae	Leaf	22.12 ± 0.68	28.65 ± 3.57	No synergy
44	<i>Xylocarpus granatum</i> J. Koenig.	Meliaceae	Pericarp	52.39 ± 3.48	53.27 ± 1.91	No synergy
			Seed	44.27 ± 5.13	54.55 ± 3.66	No synergy

Percentage of growth inhibition of novobiocin against *A. baumannii* ATCC 19606 was 6.67%.

^aPercentage of growth inhibition in the present of plant extract (PE) and plant extract in combination with novobiocin (PE+NOV) against *A. baumannii* ATCC 19606.
^bSD Standard Deviation.

^cSynergy: (PE + NOV) > (PE) + (NOV); No synergy: (PE + NOV) < (PE) + (NOV) [6].

*P < 0.01: Significantly different from the effect of plant extract.

tobramycin and resistant to amikacin, ampicillin, azithromycin, erythromycin, and gentamicin which conducted by disc diffusion method [11]. Well-isolated colonies of *A. baumannii* ATCC 19606 were grown in Mueller Hinton Broth (MHB) (Difco Laboratories, Detroit, MI) at 37°C for 18–24 h. The culture density was adjusted to McFarland standards No. 0.5 and resuspended in MHB to obtain a final concentration of 1×10^6 cfu/ml.

Medicinal plant materials

Tested medicinal plants are shown in Table 1. Fifty-one ethanol extracts of 44 Thai medicinal plant species were kindly provided by the Natural Products Research Center, Prince of Songkla University, Hat Yai, Thailand [12]. Collected plant materials were washed with distilled water and dried at 60°C overnight. Ground plant material was macerated with 95% ethanol (1:2 w/v) for 7 days. The extract was filtered and evaporated using rotary evaporator at 45°C until it became completely dry. A stock solution (200 mg/ml) was prepared by dissolving 0.2 g of the dried extract in 1 ml of dimethylsulfoxide (DMSO) (Merck, Germany) and stored at -20°C.

Determination of minimum inhibitory concentration (MIC) of novobiocin

The MIC of novobiocin was determined by the broth microdilution method as described by the Clinical and Laboratory Standard Institute (CLSI) [13].

Intrinsic antibacterial activity and resistant modifying ability of medicinal plant extracts

Intrinsic antibacterial activities were determined by growth inhibition assays [9]. The bacterial culture (100 µl) was inoculated into a 96-well microtiter plate containing 50 µl of crude extracts (1,000 µg/ml) and 50 µl of MHB and then incubated at 37°C for 18 h. The intrinsic antibacterial activity was exhibited as the percentage of growth inhibition and calculated from the following equation:

$$\% \text{Growth inhibition} = [(OD_A - OD_B) \times 100] / OD_A$$

Where OD_A is Optical density (OD) 595 nm of bacteria culture in MHB supplemented with 1%DMSO as positive control and OD_B is OD 595 nm of the bacterial culture in MHB supplemented with plant extracts.

Resistant modifying ability of the extracts was observed by adding of 50 µl novobiocin at a concentration of 1/8xMIC (1 µg/ml) into the tested plate instead of MHB. This biological activity was exhibited as the percentage of growth inhibition as well but calculated from the following equation, where OD_C is OD 595 nm of the bacterial culture in MHB supplemented with the plant extract in combination with novobiocin:

$$\% \text{Growth inhibition} = [(OD_A - OD_C) \times 100] / OD_A$$

Effective medicinal plants that demonstrated a synergistic effect with novobiocin and exhibited bacterial growth

inhibition more than 90% were selected for further experiments. The efficacy of combination therapy of the promising medicinal plants with novobiocin was additionally determined by measuring the resistant modifying capabilities of the extracts at varying concentrations ranging from 7.8 to 250 µg/ml.

Phytochemical screening methods

Phytochemical screening tests for alkaloids, condensed tannins, flavonoids, hydrolysable tannins, steroids, and triterpenes were qualitatively analyzed by standard colour tests as previously described [14].

Results and discussion

Intrinsic resistance of *A. baumannii* to novobiocin was observed with MIC value at 8 µg/ml. As shown in Table 1, 48 out of 51 tested ethanol extracts at concentration of 250 µg/ml had low inherent antibacterial activity (% of bacterial growth inhibition was less than 80%). In combination with the antibiotic, the extracts of 18 medicinal plants demonstrated synergistic interaction against *A. baumannii*. Interestingly, the bacterial growth inhibition in the presence of novobiocin in combination with the extracts of *Holarrhena antidysenterica*, *Punica granatum*, *Quisqualis indica*, *Terminalia bellirica*,

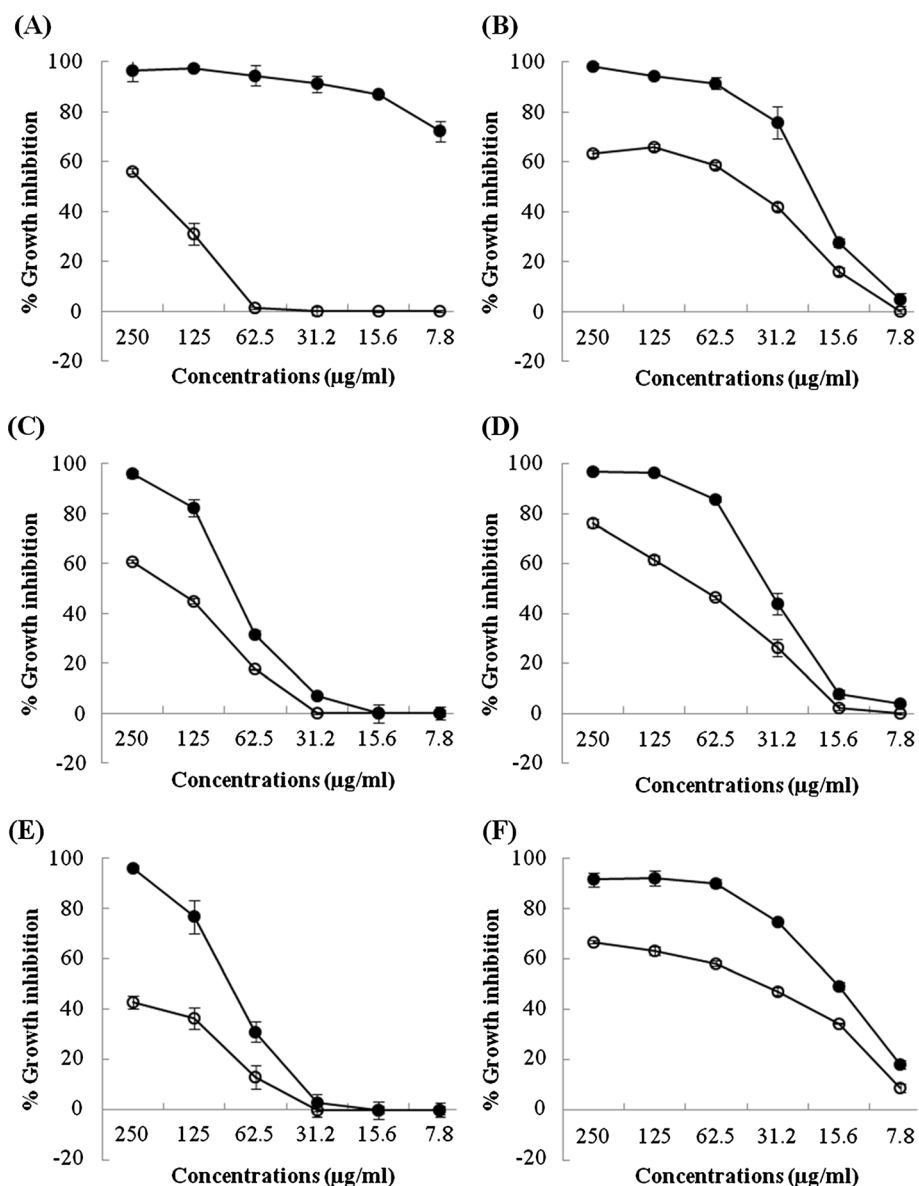


Figure 1 Bacterial growth inhibition of *Holarrhena antidysenterica* (A), *Punica granatum* (B), *Quisqualis indica* (C), *Terminalia bellirica* (D), *Terminalia chebula* (E), and *Terminalia sp.* (F) ethanol extracts (○) and the extracts in combination with 1/8xMIC of novobiocin (●) against *Acinetobacter baumannii* ATCC 19606. Percentage of bacterial growth inhibition of 1/8xMIC of novobiocin on this pathogen was 6.67%.

Table 2 Extraction yields and phytochemical constituents of tested medicinal plant extracts

	Botanical names	Part used	Yield (%; w/w) ^a	Phytochemical constituents ^b					
				1	2	3	4	5	6
1	<i>Aegle marmelos</i> (L.) Corr. Serr.	Fruit	5.3	+	+	+	-	+	-
2	<i>Ardisia colorata</i> Roxb.	Fruit	4.4	+	+	-	-	+	-
3	<i>Asclepias curassavica</i> L.	Wood	0.9	+	+	-	-	-	-
4	<i>Centella asiatica</i> (L.) Urb.	Whole	6.0	+	-	-	-	+	-
5	<i>Cinnamomum bejolghota</i> (Buch.-Ham.) Sweet	Wood	2.2	+	+	-	-	+	-
		Bark	14.6	+	-	-	+	+	-
6	<i>Cinnamomum porrectum</i> (Roxb.) Kosterm.	Wood	11.2	-	-	-	-	+	-
		Bark	7.0	+	+	-	-	+	-
7	<i>Curcuma longa</i> L.	Rhizome	13.9	+	+	+	-	+	-
8	<i>Curcuma zedoaria</i> (Christm.) Roscoe	Rhizome	13.9	+	+	+	-	-	+
9	<i>Derris scandens</i> Benth.	Stem	3.2	-	+	-	-	+	-
10	<i>Dracaena loureiri</i> Gagnep.	Wood	16.9	-	-	-	-	-	+
11	<i>Dryopteris symratica</i> (Willd.) Kuntze	Stem	4.5	+	+	-	-	+	-
12	<i>Eleutherine americana</i> (Aubl.) Merr. ex K.	Bulb	4.8	+	+	-	-	-	-
13	<i>Euphorbia thymifolia</i> L.	Whole plant	1.3	-	+	-	-	+	-
14	<i>Garcinia mangostana</i> L.	Pericarp	5.3	-	-	-	-	-	-
15	<i>Gymnopetalum cochinchinensis</i> (Lour.) Kurz	Fruit	7.6	-	-	-	-	+	-
16	<i>Holarrhena antidiysenterica</i> (L.) Wall. ex A. DC.	Bark	2.1	+	+	-	-	-	+
17	<i>Impatiens balsamina</i> L.	Stem	5.2	-	+	-	-	+	-
18	<i>Manilkara achras</i> (Mill.) Fosb.	Fruit	26.7	+	-	+	-	-	+
19	<i>Millingtonia hortensis</i> L.f.	Flower	25.4	+	+	+	-	-	-
20	<i>Mitragyna speciosa</i> Korth.	Leaf	5.9	+	+	-	-	+	-
21	<i>Momordica charantia</i> L.	Vine	3.0	+	-	-	-	+	-
22	<i>Morinda citrifolia</i> L.	Fruit	7.3	+	-	+	-	+	-
23	<i>Murdannia loriformis</i> (Hassk) R. Rao & Kammathy	Whole plant	7.6	+	-	-	-	+	-
24	<i>Oroxylum indicum</i> (L.) Vent.	Leaf	3.7	+	+	-	-	+	-
25	<i>Peltophorum pterocarpum</i> (DC.) Backer ex K. Heyne	Flower	7.1	+	-	-	-	-	-
		Bark	7.1	+	+	-	-	-	+
26	<i>Piper betle</i> L.	Leaf	12.4	-	+	-	-	+	-
27	<i>Piper nigrum</i> L.	Fruit	4.2	+	-	-	-	+	-
		Seed	4.2	+	-	-	-	+	-
28	<i>Piper retrofractum</i> Vahl	Fruit	7.0	-	-	-	-	+	-
29	<i>Piper sarmentosum</i> Roxb	Leaf	1.7	+	-	-	-	+	-
30	<i>Pluchea indica</i> (L.) Less.	Leaf	17.8	+	+	-	-	+	-
31	<i>Psidium guajava</i> L.	Leaf	8.0	+	+	-	-	+	-
32	<i>Punica granatum</i> L.	Pericarp	13.0	+	+	+	-	-	+
33	<i>Quercus infectoria</i> G.Olivier	Gall	37.8	+	-	-	+	-	-
34	<i>Quisqualis indica</i> L.	Flower	11.0	+	-	+	+	+	-

Table 2 Extraction yields and phytochemical constituents of tested medicinal plant extracts (Continued)

35	<i>Rhizophora mucronata</i> Lam.	Fruit	10.7	+	+	-	-	-	+
		Bark	11.6	-	+	-	-	-	+
36	<i>Rhodomirtus tomentosa</i> (Aiton) Hassk.	Stem	7.1	+	+	-	-	-	+
37	<i>Sandoricum indicum</i> Cav.	Root	4.0	+	-	-	-	+	-
38	<i>Tamarindus indica</i> L.	Leaf	4.8	+	+	+	-	+	-
39	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Fruit	14.8	+	-	-	-	+	-
40	<i>Terminalia chebula</i> (Gaertn.) Retz.	Fruit	5.9	+	+	-	-	-	+
41	<i>Terminalia</i> sp.	Fruit	23.9	+	-	-	+	-	-
42	<i>Theobroma cacao</i> L.	Pericarp	3.6	+	+	-	-	+	-
		Seed	5.9	-	+	+	-	-	+
43	<i>Vitex trifolia</i> L.	Leaf	ND ^c	+	+	-	-	+	-
44	<i>Xylocarpus granatum</i> J. Koenig.	Pericarp	2.6	+	+	-	-	+	-
		Seed	6.7	+	+	+	-	-	+

^aPercentage extract yields of medicinal plants were weight of crude extract per 100 g of dried plant materials.

^bPhytochemical constituents: 1, alkaloids; 2, condensed tannins; 3, flavonoids; 4, hydrolysable tannins; 5, steroids and 6, triterpenoids; '-' indicates absence of phytoconstituents '+' indicates presence of phytoconstituents.

^cND Not determined.

Terminalia chebula, and *Terminalia* sp. extracts was significantly higher than the intrinsic antibacterial activity of the extracts (Table 1).

To explore the potential of developing a more powerful combination therapy of these medicinal plants with novobiocin, we determined the resistant modifying ability of varying concentrations of the extracts from 7.8 to 250 µg/ml by growth inhibition assay as illustrated in Figure 1. *Holarrhena antidysenterica* extract which concentrations ranging from 7.8 to 62.5 µg/ml possessed no intrinsic anti-acinetobacter activity (Figure 1A) was demonstrated to be a powerful RMA in combination with novobiocin against this pathogen.

Our preliminary phytochemical test revealed that alkaloids were common principles among the effective extracts. In addition to alkaloids, other compounds including condensed tannins, triterpenoids, flavonoids, hydrolysable tannins, and steroids were detected (Table 2). Although the antibiotic resistant modifying ability of active principles of the effective medicinal plants has never been investigated, plant-derived alkaloids have been well-clarified as efflux pump inhibitors (EPIs) for Gram positive bacteria [15,16]. Recent evaluation of 13 phyto-alkaloids for their EPI potential against staphylococcal isolates revealed that 60% and 30% of the tested compounds exhibited the activity against methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA), respectively [16]. Four plant-derived alkaloids consisting of reserpine, quinine, harmaline, and piperine possessed notable potential EPI

activities on both MRSA and MSSA [16]. More importantly, their mechanisms of actions as a RMA have been proposed. Piperine was recorded as an inhibitor of MdeA [17] and NorA [18] efflux pumps of *S. aureus* and Rv1258c efflux pump of *Mycobacterium tuberculosis* [19]. Reserpine was found as an inhibitor of Bmr efflux pump in *Bacillus subtilis*, Tet(K) and NorA efflux pumps of *S. aureus* [20]. In addition to phyto-alkaloids, several plant-derived polyphenols such as epigallocatechin gallate of *Camellia sinensis*, tellimagrandin I and rugosin B isolated from *Rosa canina* have been established as useful RMAs with different mechanisms of actions including inhibitions of adapted drug target sites or enzymatic degradation of drugs [4]. Intensive investigations on plant-derived compounds as RMAs have been performed in Gram-positive, but relatively very few studies have been carried out to evaluated RMA activities of plant-derived compounds on Gram-negative bacteria [21-23].

In the last decade multidrug resistance in *A. baumannii* became a serious growing problem worldwide. Colistin, an old antibiotic with risk toxicity, has recently been brought back into use to treat MDR bacteria as a stopgap measure until new antibiotics can be developed [24]. A number of workers have proposed the synergistically action combination of conventional antibiotics with RMA act synergistically against MDR Gram-negative bacteria [4,25,26]. We have demonstrated that certain plant ethanol extracts significantly enhanced the activity of novobiocin against *A. baumannii*. *Holarrhena antidysenterica* is of interest

since the extract at 7.8 to 62.5 µg/ml possessed no intrinsic antibacterial activity, but in combination with sub-MIC of novobiocin led to a marked decrease in the bacterial growth. Alkaloids were proposed as active principles of the plant that possessed antibacterial activity on *S. aureus*, *S. epidermidis*, *Streptococcus faecalis*, *B. subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [27-29]. Some of the alkaloids such as pubadysone, pubescine, norholadiene, pubescimine, puboestrene, pubamide, and naringenin was isolated from bark, seeds, and leaves of this plant [30-32].

Our previous investigation demonstrated that ellagic acid which acts as an efflux pump inhibitor exhibited a synergistic effect with novobiocin and other aminocoumarins against both *A. baumannii* ATCC 19606 and MDR *A. baumannii* [9]. Ethylenediaminetetraacetic acid and polyethyleneimine that disturb outer membrane permeability have been reported as RMA for novobiocin against *P. aeruginosa* and *Stenotrophomonas morelense* [33,34]. Similarly, berry-derived phenolic compounds that efficiently destabilized outer membrane permeability resulted in increase in novobiocin susceptibility of *Salmonella enterica* serotype Typhimurium [35].

Since intrinsic novobiocin resistance in *A. baumannii* is related to the synergistic interaction between limited outer membrane permeability and energy-dependent multidrug efflux pumps [36,37], the RMA for novobiocin possibly acts as a permeabilizer and/or an efflux pump inhibitor.

Conclusion

The RMA activity of Thai medicinal plants in combination with novobiocin against *A. baumannii* is reported for the first time. These findings led us to the development of a new generation of phytopharmaceuticals that using plant-derived compounds in combination with existing antibiotics to treat MDR *A. baumannii* that currently are almost untreatable. Its mechanism of action as well as the active constituents of a promising plant, *Holarrhena antidysenterica* should be further investigated.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the Thailand research Fund-the Commission on Higher Education (MRG 5480069, Fiscal year 2011-2013) and the Higher Education Research Promotion and National Research University of Thailand, Office of the Higher Education Commission.

Author' contributions

PN designed and carried out the study. SC and SV supervised in the design of the study and contributed to the writing process. All authors read and approved the final manuscript.

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Received: 19 March 2012 Accepted: 26 April 2012

Published: 26 April 2012

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doi:10.1186/1472-6882-12-56

Cite this article as: Phatthalung *et al.*: Thai ethnomedicinal plants as resistant modifying agents for combating *Acinetobacter baumannii* infections. *BMC Complementary and Alternative Medicine* 2012 **12**:56.

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