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.

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**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...
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 ± SD | Interpretation |
|----------------------------------|---------------------|-----------|--------------------------|----------------|
| 1 Aegle marmelos (L.) Corr. Serr.| Rutaceae            | Fruit     | 22.10 ± 0.68             | No synergy     |
| 2 Ardisia colorata Roxb.         | Primulaceae         | Fruit     | 30.17 ± 2.56             | Synergy        |
| 3 Asclepias curassavica L.       | Asclepiadaceae      | Wood      | 40.81 ± 0.28             | No synergy     |
| 4 Centella asiatica (L.) Urb.    | Apiaceae            | Whole     | 19.09 ± 1.06             | No synergy     |
| 5 Cinnamomum bejolghota (Buch.-Ham.) Sweet | Lauraceae   | Wood     | 58.84 ± 1.37             | No synergy     |
|                                  |                     | Bark      | 55.62 ± 4.98             | No Synergy     |
| 6 Cinnamomum porrectum (Roxb.) Kosterm. | Lauraceae | Wood     | 29.72 ± 6.54             | No synergy     |
| 7 Curcuma longa L.               | Zingiberaceae       | Rhizome   | 86.91 ± 2.64             | No synergy     |
| 8 Curcuma zedoaria (Christm.) Roscoe | Zingiberaceae | Rhizome   | 77.73 ± 0.48             | No synergy     |
| 9 Demis scandens Benth.          | Leguminosea         | Stem      | 49.01 ± 2.37             | No synergy     |
| 10 Draecaena lourei Gagnep.      | Agavaceae           | Wood      | 30.08 ± 0.99             | No synergy     |
| 11 Dryopteris symnatica (Wild.) Kunz) | Dryopteridaceae     | Stem      | 17.59 ± 0.41             | Synergy        |
| 12 Eleutherine americana (Aubl.) Merr. ex K. | Iridaceae | Bulb     | 17.87 ± 1.89             | No synergy     |
| 13 Euphorbia thymifolia L.       | Euphorbiaceae       | Whole plant | 53.64 ± 0.90       | Synergy        |
| 14 Garcinia mangostana L.        | Clusiaceae          | Pericarp  | 93.25 ± 3.65             | No synergy     |
| 15 Gymnopetalum cochinchinensis (Lour.) Kurz) | Cucurbitaceae | Fruit     | 26.17 ± 0.59             | No synergy     |
| 16 Holanthena antidyseyntica (L.) Wall. ex A. DC. | Apocynaceae | Bark     | 65.88 ± 0.11             | Synergy        |
| 17 Impatiens balsamina L.        | Balsaminaceae       | Stem      | 9.77 ± 0.30              | No synergy     |
| 18 Manilkara achars (Mill) Fosb. | Sapotaceae          | Fruit     | 56.59 ± 1.02             | No synergy     |
| 19 Millingtonia hortensis L.f.   | Bignoniaceae        | Flower    | 28.97 ± 4.30             | Synergy        |
| 20 Mitragyna speciosa Kor.       | Rubiaceae           | Leaf      | 43.33 ± 2.40             | Synergy        |
| 21 Momordica charantia L.        | Cucurbitaceae       | Vine      | 22.26 ± 0.85             | No synergy     |
| 22 Morinda citrifolia L.         | Rubiaceae           | Fruit     | 16.96 ± 0.63             | Synergy        |
| 23 Murdannia lanifarm (Hassk.) R. Rao & Kammathy | Commelinaceae | Whole plant | 16.42 ± 1.51          | No synergy     |
| 24 Oroxylum indicum (L.) Vent.   | Bignoniaceae        | Leaf      | 67.18 ± 1.59             | No synergy     |
| 25 Peltophorum pterocarpm (DC.) Backer ex K. Heyne | Fabaceae         | Flower    | 42.80 ± 0.43             | No synergy     |
|                                  |                     | Bark      | 78.26 ± 0.60             | Synergy        |
| 26 Piper betle L.                | Piperaceae          | Leaf      | 42.72 ± 0.13             | No synergy     |
| 27 Piper nigrum L.               | Piperaceae          | Fruit     | 38.07 ± 1.96             | No synergy     |
|                                  |                     | Seed      | 29.07 ± 0.75             | No synergy     |
| 28 Piper retrofractum Vahl       | Piperaceae          | Fruit     | 44.02 ± 1.08             | No synergy     |
| 29 Piper sarmentosum Roxb.       | Piperaceae          | Leaf      | 20.70 ± 0.88             | No synergy     |
| 30 Pluchea indica (L.) Less.     | Asteraceae          | Leaf      | 26.64 ± 0.97             | Synergy        |
| 31 Psidium guajava L.            | Myrtaceae           | Leaf      | 71.24 ± 2.00             | Synergy        |
| 32 Punica granatum L.            | Puniceaceae         | Pericarp  | 72.58 ± 1.20             | Synergy        |
| 33 Quercus infectoria G.Olivier  | Fagaceae            | Gall      | 89.09 ± 0.15             | No synergy     |
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 × 10⁶ 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:

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

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 1/8×MIC (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:

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

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

### 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)

| NO | Scientific name               | Family          | Part               | Intrinsic activity (PE) | Resistant modifying activity (PE + NOV) |
|----|--------------------------------|-----------------|--------------------|-------------------------|----------------------------------------|
| 34 | *Quisqualis indica* L.        | Combretaceae    | Flower             | 79.22 ± 0.28            | 94.63 ± 2.62* Synergy                  |
| 35 | *Rhizophora mucronata* Lam.  | Rhizophoraceae  | Fruit              | 44.64 ± 0.59            | 53.35 ± 2.56 Synergy                   |
| 36 | *Rhodomyrtus tomentosa* (Aiton) Hassk. | Myrtaceae | Bark               | 42.68 ± 8.20            | 53.03 ± 4.95 Synergy                   |
| 37 | *Sandonicum indicum* Cav.     | Meliaceae       | Root               | 65.24 ± 1.32            | 66.94 ± 2.13 No synergy                |
| 38 | *Tamandanus indica* L.        | Fabaceae        | Leaf               | 19.76 ± 1.55            | 25.03 ± 3.45 No synergy                |
| 39 | *Terminalia bellirica* (Gaertn.) Roxb. | Combretaceae | Fruit              | 74.79 ± 0.53            | 95.68 ± 1.14* Synergy                  |
| 40 | *Terminalia chebula* (Gaertn.) Retz. | Combretaceae | Fruit              | 61.25 ± 0.42            | 94.33 ± 1.95* Synergy                  |
| 41 | *Terminalia sp.*              | Combretaceae    | Fruit              | 79.53 ± 0.24            | 95.92 ± 1.10* Synergy                  |
| 42 | *Theobroma cacao* L.          | Sterculiaceae   | Pericarp           | 17.35 ± 0.74            | 22.81 ± 0.68 No synergy                |
| 43 | *Vitex trifolia* L.           | Verbenaceae     | Leaf               | 19.25 ± 1.08            | 29.61 ± 4.13 Synergy                   |
| 44 | *Xylocarpus granatum* J. Koenig. | Meliaceae      | Pericarp           | 52.39 ± 3.48            | 53.27 ± 1.91 No synergy                |

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

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

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

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

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.
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.

**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, Terminalia chebula, and Terminalia sp.* 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%.

**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].

![Figure 1](http://www.biomedcentral.com/1472-6882/12/56)
| Botanical names | Part used                  | Yield (%; w/w) | Phytochemical constituents |   |   |   |
|----------------|---------------------------|----------------|----------------------------|---|---|---|
| Aegle marmelos (L.) Corr. Serr. | Fruit                     | 5.3            | +                          | + | + | - |
| Ardisia colorata Roxb.           | Fruit                     | 4.4            | +                          | + | - | - |
| Asclepias curassavica L.         | Wood                      | 0.9            | +                          | + | - | - |
| Centella asiatica (L.) Urb.      | Whole                     | 6.0            | +                          | - | - | + |
| Cinnamomum bejolghota (Buch.-Ham.) Sweet | Wood                  | 2.2            | +                          | + | - | - |
| Cinnamomum porrectum (Roxb.) Kosterm. | Wood                  | 11.2           | -                          | - | - | + |
| Curcuma longa L.                 | Rhizome                   | 13.9           | +                          | + | + | - |
| Curcuma zedoaria (Christm.) Roscoe | Rhizome                 | 13.9           | +                          | + | + | - |
| Dioscorea scandens Benth.        | Stem                      | 3.2            | -                          | + | - | - |
| Datura stramonium (L.)          | Whole plant               | 6.0            | +                          | - | - | + |
| Dryopteris sympatica (Wild.) Kuntze | Stem                  | 4.5            | +                          | + | - | - |
| Eleutherine americana (Aubl.) Merr. ex K. | Bulb            | 4.8            | +                          | + | - | - |
| Euphorbia thymifolia L.         | Whole plant               | 1.3            | -                          | + | - | - |
| Garcinia mangostana L.          | Pericarp                  | 5.3            | -                          | - | - | - |
| Gymnopedium cochinichinensis (Lour.) Kurz | Fruit             | 7.6            | -                          | - | - | + |
| Holarthina antidysenterica (L.) Wall. ex A. DC. | Bark                  | 2.1            | +                          | + | - | - |
| Impatiens balsamina L.          | Stem                      | 5.2            | -                          | + | - | - |
| Manilkara acha (Mill.) Fosb.     | Fruit                     | 26.7           | +                          | - | + | - |
| Millingtonia hortensis L.f.      | Flower                    | 25.4           | +                          | + | - | - |
| Mitragyna speciosa Korth.        | Leaf                      | 5.9            | +                          | + | - | - |
| Momordica charantia L.          | Vine                      | 3.0            | -                          | + | - | - |
| Morinda citrifolia L.           | Fruit                     | 7.3            | +                          | - | + | - |
| Murdannia ionifera (Hassk.) R. Rao & Kammathy | Whole plant | 7.6            | +                          | - | - | - |
| Oroxyllum indicum (L.) Vent.     | Leaf                      | 3.7            | +                          | + | - | - |
| Peltophorum pterocarpum (DC.) Backer ex K. Heyne | Flower     | 7.1            | +                          | - | - | - |
| Piper betle L.                   | Leaf                      | 12.4           | +                          | - | - | + |
| Piper nigrum L.                  | Fruit                     | 4.2            | +                          | - | - | + |
| Piper retrofractum Vahl         | Fruit                     | 7.0            | -                          | - | + | - |
| Piper sarmentosum Roxb           | Leaf                      | 1.7            | +                          | - | - | + |
| Pluchea indica (L.) Less.        | Leaf                      | 17.8           | +                          | + | - | - |
| Psidium guajava L.               | Leaf                      | 8.0            | +                          | + | - | - |
| Punica granatum L.               | Pericarp                  | 13.0           | +                          | + | - | - |
| Quercus infectoria G.Olivier    | Gall                      | 37.8           | +                          | - | + | - |
| Quisqualis indica L.             | Flower                    | 11.0           | +                          | - | + | + |
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, 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 sinesis*, 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

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

| No. | Medicinal Plant | Part | Extraction Yields (%) | Alkaloids | Condensed Tannins | Flavonoids | Hydrolysable Tannins | Steroids | Triterpenoids | Others |
|-----|----------------|------|-----------------------|-----------|-------------------|-----------|----------------------|----------|--------------|--------|
| 35  | *Rhizophora mucronata* Lam. | Fruit | 10.7 | + | + | - | - | - | + |
|     | Bark            |      | 11.6 | - | + | - | - | - | + |
| 36  | *Rhodomysurus tamentosa* (Aiton) Hassk. | Fruit | 7.1 | + | + | - | - | - | + |
| 37  | *Sandoricum indicum* Cav. | Root | 4.0 | + | - | - | - | + | - |
| 38  | *Tamarindus indica* L. | Leaf | 4.8 | + | + | + | - | + | - |
| 39  | *Terminalia bellirica* (Gaertn.) Roxb. | Fruit | 14.8 | + | - | - | - | + | - |
| 40  | *Terminalia chebula* (Gaertn.) Retz. | Fruit | 5.9 | + | + | - | - | - | + |
| 41  | *Terminalia sp.* | Fruit | 23.9 | + | - | - | + | - | - |
| 42  | *Theobroma cacao* L. | Pericarp | 3.6 | + | + | - | - | - | + |
|     | Seed            |      | 5.9 | - | + | + | - | - | + |
| 43  | *Vitex trifolia* L. | Leaf | ND² | + | + | - | - | + | - |
| 44  | *Xylocarpus granatum* J. Koenig. | Pericarp | 2.6 | + | + | - | - | + | - |
|     | Seed            |      | 6.7 | + | + | - | - | - | + |

*Percentage extract yields of medicinal plants were weight of crude extract per 100 g of dried plant materials.

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

²ND Not determined.
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 form 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 morosele* [33,34]. Similarly, berry-derived phenolic compounds that efficiently destabilized outer membrane permeability resulted in increase in novobiocin susceptibility of *Salmonella enterica* serotype Typhinimum [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.

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**Author’s 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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