Original Article

Evaluation of Levofloxacin and Yahom-Navakot Remedy Extract Combination Therapy Against Antibiotic Resistant Bacteria *In Vitro*

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Abstract

Introduction: Antibiotic resistant bacteria are being considered a serious public health challenge. Levofloxacin is a broad-spectrum antibiotic, developed to replace previously resistant antimicrobials. The use of natural products as an antimicrobial drug is an alternative way to reduce bacterial resistance by synthesized drugs and enhance their efficacy. Yahom-Navakot (YN) is a Thai remedy in the Thai essential drug list. The indications for YN treatment are to relieve fatigue, dizziness, malaise, and vomiting after alleviation of fever. Previous studies have reported antibiotic enhancing effects of YN. This study was aimed to evaluate the antibacterial activities of levofloxacin and Yahom-Navakot Remedy Extract.

Methods: YN remedy was macerated with 95% ethanol, after solvent evaporation the extract was combined with levofloxacin (1:1). By using modified resazurin in the broth micro-dilution assay, the antibacterial activities of the combination were evaluated against the pathogenic bacteria; *Staphylococcus aureus* (DMST 20651), Methicillin-Resistant *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC BAA 2789), *Salmonella typhi* (DMST 22842), *Shigella dysenteriae* (DMST 15111), and *Escherichia coli* (ATCC 25922). *P. aeruginosa,* MRSA, *S. typhi, S. dysenteriae*, and *E. coli* were antibiotic resistant bacteria strains according to WHO criteria. Levofloxacin was used as a control in this study.

Results: *In vitro* YN extract showed potent antibacterial effect against *S. aureus* (MIC = 0.625, MBC = 0.39 mg/mL), MRSA (MIC = 0.625, MBC = 25 mg/mL), and *S. epidermidis* (MIC = 0.3125, MBC = 50 mg/mL). YN extract (At MICs against selected bacteria strains) demonstrated enhanced antibacterial activities of levofloxacin against MRSA (MIC < 3×10^{-8}, MBC < 3×10^{-8} µg/mL) and *S. epidermidis* (MIC = 0.048, MBC = 25 µg/mL) as determined by reduced MICs and MBCs. However, it did not affect other selected bacteria strains; *P. aeruginosa*, *K. pneumoniae*, *S. typhi, S. dysenteriae*, and *E. coli* (MIC > 5 mg/mL).

Conclusions: YN showed evidence of antibacterial activity. It had potential antibiotic enhanced activity with levofloxacin; however, further *in vivo* and clinical studies are essential to evaluate YN's efficacy and safety.

Keywords: Yahom-Navakot remedy, Levofloxacin, Bacterial antibiotic resistance, Antibacterial activities

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Introduction

Antibiotic resistance bacteria is a serious public health challenge caused by the overuse of antibiotics, inappropriate prescriptions, extensive use in agriculture, unavailability and expense of new antibiotics, and regulatory barriers.[1] MRSA (Methicillin-resistant *Staphylococcus aureus*) infection is one of the causes of nosocomial infections and an important unsolved problem in health systems. It can cause skin and soft tissue infections, bone and joint infection, pneumonia, bacteremia, and endocarditis.[2] The prevalence of MRSA infection differs around the world. The range of invasive MRSA isolated in Europe was between 0.9% to 56%.[3] When compared with *S. aureus* infections, MRSA infection was found between 13 to 74%.[4] Several studies indicate prevalence of MRSA in Thailand.[5, 6] From 2009 - 2014, MRSA prevalence among *S. aureus* bacteremia cases was 10% in rural Thailand.[5] One of the studies reported that 17% of MRSA infection was evident from patients who attended outpatient and inpatient departments from January to December 2017 at King Chulalongkorn Memorial Hospital, a tertiary care center and a university hospital in Bangkok, Thailand in 2017.[6] Comorbidities significantly associated with MRSA were cardiovascular, neurological, and chronic lung diseases.[6]

It is imperative to judiciously prescribe appropriate antibiotics for treatment of infections. Many generations of broad-spectrum antibiotics have been developed. Levofloxacin is one of the new generation antibiotics and is a broad-spectrum, third-generation fluoroquinolone antibiotic. It is an antibiotic of choice to treat respiratory infection, especially; nosocomial pneumonia, urinary tract infection, and skin infections.[7] According to many *in vitro* reports, levofloxacin showed better effectiveness against some drug-resistant bacteria such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) than other members of the fluoroquinolone group.[8, 9] However, the high dose and volume of prescription and overuse may cause many side effects with patients including antibiotic resistant problems. Accordingly, the World Health Organization (WHO) has responded to this problem by suggesting a global action plan on antimicrobial resistance to reduce bacterial resistant problems.[10] Studies on how to enhance the antibiotic effect and reduce the overuse of antibiotics were amongst the WHO's strategies outlined.

Yahom-Navakot (YN) is a well known and widely herbal remedy used in Thailand. Its properties are suggested to relieve dizziness, malaise, and vomiting, especially; to treat Lom Plai Khai (The symptoms after fever decreasing such as body pain, debility, dizziness, and flatulence). From the analysis of YN's ingredients (show in Table 1), some of the ingredients are known to have antibacterial effects and antibiotic-enhancing effects, such as *Zingiber officinale* Roscoe, *Glycyrrhiza glabra* L., and *Carum carvi* L.[14] Moreover, 43 out of the 55 herbal ingredients of YN have demonstrated antibacterial activities against gram positive and gram negative bacteria. Bacteria examined to be susceptible to YN include *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae,* and *Escherichia coli*. Only 5 herbs were active against Methicillin-Resistant *Staphylococcus aureus* (MRSA). These were the extracts of *Eleusine indica, Myristica fragrans, Piper sarmentosum, Pogostemon cablin,* and *Terminalia bellirica* (Table 2).

Many investigations using natural products to reduce resistant-bacterial strains, side effects of antibiotics, and enhancing antibiotics effect have been undertaken.[11] There were several studies about the combinations of antibiotics and natural products, for example, the study of the combination of Chinese herbal medicine and antibiotics on extensively drug-resistant enterobacteria and non-fermentative bacterial infection[12] and the study of the combination effects of ethanolic extract of buffalo thorn (*Ziziphus mucronata Willd. subsp. mucronata Willd.*)[13] and antibiotics against clinically essential bacteria.[8]

There were several studies using combinations of antibiotics with many herbal ingredients in YN (Table 3). The seeds of *Carum carvi* were shown to enhance the bioavailability of cefdinir (89%) and cloxacillin (100%).[14] *Cuminum cyminum* seeds provided antibiotic bioenhancing activity in the range of 25 - 335%.[14] Glycyrrhizin; the main chemical component of *Glycyrrhiza glabra,* enhanced bioactivity of rifampicin, ampicillin, tetracycline, and nalidixic acids against *M. smegmatis, B. subtilis,* and *E. coli*.[14] Methanol and hexane extracts of

Nigella sativa L. could increase the permeation of amoxicillin significantly ($P < 0.001$) as compared to the control.[67] The extract of *Pimpinella anisum* seeds showed synergism with cephradine against *S. pneumonia* and *S. aureus* as evaluated using the disc diffusion method.[68] Sophoraflavanone B; the main chemical constituent from *Sophora tomentosa* L., markedly reduced the MICs of the β-lactam antibiotics; ampicillin (AMP) and oxacillin, aminoglycosides gentamicin, quinolones ciprofloxacin and norfloxacin against *S. aureus*; MRSA.[69] The essential oil from *Trachyspermum ammi* can reduce the MICs and inhibition zone of gentamicin against *S. aureus*; MRSA.[70] Ginger enhanced the bioavailability of rifampicin by 65%, ethionamide by 56% and Azithromycin by 78% (Table 3). The results of these studies, supported the possibility of using traditional medicines including herbs to enhance the therapeutic effects of antibiotics.

Analysis of YN's ingredients suggests further study of YN as an antibiotic enhancer is scientifically prudent. There has been no research published to date on this issue. This study was aimed to evaluate the combined antibacterial activities using levofloxacin and YN extract.

Table 1 Yahom-Navakot Remedy's Ingredients

No.	Scientific names	Thai names	Family names	Used Parts	Taste	Traditional Use	Proportion in Remedy All portion = 211
1	*Alyxia reinwardtii* Bl.	Cha-lood	APOCYNACEAE	Bark	Fragrant	Carminative, analeptic, cardiotonic	4
2	*Amomum krevanh* Pierre ex Gagnep.	Kra-waan	ZINGIBERACEAE	Fruit	Spicy	Carminative, analeptic	4
3	*Anethum graveolens* L.	Tien-ta-tak-ka-taen	APIACEAE	Seed	Bitter	Carminative, analeptic, digestive	4
4	*Angelica dahurica* Fisch. ex Hoffm.	Kote-so	APIACEAE	Root	Spicy	Antipyretic, antitussis	4
5	*Angelica sinensis* (Oliv.) Diels	Kote-chiang	APIACEAE	Root	Sweet	Antipyretic, antitussis	4
6	*Aquilaria crassna* Pierre	Krit-sa-na	THYMELAEACEAE	Wood	Fragrant	Analeptic, cardiotonic, haematinic, antipyretic	4
7	*Artemisia annua* L.	Kote-chu-la-lam-pha	ASTERACEAE	Leaf	Bitter	Antipyretic	4
8	*Atractylodes lancea* Thunb.	Kote-kha-mao	ASTERACEAE	Rhizome	Sweet	Analeptic, carminative	4
9	*Brucea javanica* (L.) Merr	Rad-cha-dud	SIMAROUBACEAE	Fruit	Bitter	Antipyretic,vermifuge, carminative, promote the appetite	4
10	*Carum carvi* L.	Tien-ta-kob	APIACEAE	Fruit	Spicy	Analeptic, antiemetic, expectorants, carminative	4
11	*Cinnamomum bejolghota* (Buch.-Ham.) Sweet	Sa-mun-waang	LAURACEAE	Bark	Spicy	Reduce dizziness, carminative	4
12	*Cinnamomum loureirii* Nees	Ob-choei-yuan	LAURACEAE	Bark	Spicy	Carminative	4
13	*Coriandrum sativum* L.	Phak-chi-la	APIACEAE	Fruit	Spicy	Carminative, antiemetic, analeptic	4
14	*Cuminum cyminum* L.	Tien-khaow	APIACEAE	Seed	Spicy	Carminative, expectorants, analeptic	4
15	*Cyperus rotundus* L.	Haeo-mu	CYPERACEAE	Rhizome	Spicy	Carminative, diuretic	4
16	*Dalbergia candenatensis* (Dennst.) Prain	Sak-khi	FABACEAE	Wood	Bitter	Antipyretic, analeptic	4

Table 1 Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Thai names	Family names	Used Parts	Taste	Traditional Use	Proportion in Remedy All portion = 211
17	*Dracaena loureiri* Gagnep.	Chan-dang	DRACAENACEAE	Wood	Bitter	Antipyretic	4
18	*Eleusine indica* (L.) Gaertn.	Ya-tin-nok	POACEAE	Tree	Bitter	Antipyretic, reduce headache	4
19	*Euphorbia antiquorum* L.	Ka-lum-pak	EUPHORBIACEAE	Wood	Fragrant	Cardiotonic	4
20	*Foeniculum vulgare* Mill.	Tien-khao-plueak	APIACEAE	Seed	Sweet	Analeptic, carminative, expectorants	4
21	*Glycyrrhiza glabra* L.	Cha-em-thet	FABACEAE	Root	Sweet	Analeptic, expectorants	4
22	*Gymnopetalum chinense* (Lour.) Merr.	Kra-dom	CUCURBITACEAE	Fruit	Bitter	Antipyretic	4
23	*Jasminum sambac* (L.) Aiton	Ma-li	OLEACEAE	Flower	Fragrant	Cardiotonic	4
24	*Kaempferia galangal* L.	Por-hom	ZINGIBERACEAE	Root	Spicy	Treat common cold, carminative, expectorants	4
25	*Lepidium sativum* L.	Tien-dang	BRASSICACEAE	Seed	Spicy	Expectorants, carminative, antiemetic, haematinic	4
26	*Ligusticum sinense* Oliv.	Kote-nua-bua	APIACEAE	Root	Bitter	Carminative, reduced headache	4
27	*Mammeas siamensis* T. Anders.	Sa-ra-phi	CALOPHYLLACEAE	Flower	Fragrant	Cardiotonic	4
28	*Mesua ferrea* L.	Bun-nak	CALOPHYLLACEAE	Flower	Fragrant	Analeptic, carminative, antipyretic, cardiotonic	4
29	*Mimusops elengi* L.	Khon-dok	SAPOTACEAE	Wood	Fragrant	Cardiotonic, reduce dizziness, antipyretic	4
30	*Mimusops elengi* L.	Phi-kun	SAPOTACEAE	Flower	Fragrant	Cardiotonic	4
31	*Myristica fragrans* Houtt.	Dok-Chan	MYRISTICACEAE	Mace	Spicy	Haematinic, analeptic, carminative	4
32	*Myristica fragrans* Houtt.	Look-Chan	MYRISTICACEAE	Seed	Spicy	Analeptic, antipyretic, carminative	4
33	*Myristica fragrans* Houtt.	Chan-ted	MYRISTICACEAE	Wood	Bitter	Antipyretic	4
34	*Nardostachys jatamansi* (D.Don) DC.	Kote-cha-da-mang-si	CAPRIFOLIACEAE	Flower	Spicy	Vermifuge, carminative	4
35	*Nelumbo nucifera* Gaertn.	Bua-luang	NELUMBONACEAE	Pollen	Fragrant	Cardiotonic, antipyretic	4

Table 1 Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Thai names	Family names	Used Parts	Taste	Traditional Use	Proportion in Remedy All portion = 211
36	*Nigella sativa* L.	Tien-dum	RANUNCULACEAE	Seed	Spicy	Expectorants, carminative, digestive, antiemetic, haematinic	4
37	*Phyllanthus emblica* L.	Ma-kham-pom	PHYLLANTHACEAE	Fruit	Sour	Expectorants, antidiarrheal, laxative	4
38	*Picrorhiza kurroa* Royle ex Benth.	Kote-kan-praw	PLANTAGINACEAE	Rhizome	Bitter	Antipyretic	4
39	*Pimpinella anisum* L.	Tien-sat-ta-but	APIACEAE	Seed	Spicy	Reduce dizziness, antipyretic, antitussis	4
40	*Pinus kesiya* Royle ex Gordon.	Son	PINACEAE	Wood	Bitter	Antipyretic, antidiarrheal	4
41	*Piper longum* L.	Di-pli	PIPERACEAE	Fruit	Spicy	Carminative, antitussis, analeptic	3
42	*Piper ribesioides* Wall.	Sa-khan	PIPERACEAE	Wood	Spicy	Carminative, analeptic	3
43	*Piper sarmentosum* Roxb.	Cha-phlu	PIPERACEAE	Leaf	Spicy	Carminative, analeptic	3
44	*Plantago ovata* Forssk.	Tien-kled-hoi	PLANTAGINACEAE	Seed	Spicy	Carminative, heamatinic	4
45	*Plumbago indica* L.	Chet-ta-mun-ploeng-daeng	PLUMBAGINACEAE	Root	Spicy	Carminative, analeptic, haemagogue	3
46	*Pogostemon cablin* (Blanco) Benth.	Pim-sen	LAMIACEAE	Matter	Fragrant	Carminative, expectorants, cardiotonic	1
47	*Aucklandia lappa* DC.	Kote-kra-duk	ASTERACEAE	Root	Bitter	Carminative, antitussis	4
48	*Astringent Sophora tomentosa* L.	Sa-ra-phat-phit	FABACEAE	Seed	Bitter	Antipyretic	4
49	*Syzygium aromaticum* (L.) Merrill & Perry	Kan-phlu	MYRTACEAE	Flower	Spicy	Carminative, expectorants	4
50	*Terminalia bellirica* (Gaertn.) Roxb.	Sa-mor-phi-phek	COMBRETACEAE	Fruit	Sour	Antipyretic, laxative	4
51	*Terminalia chebula* Retz.	Kote-phung-pla	COMBRETACEAE	Gall	Astringent	Antidiarrheal	4
52	*Tinospora crispa* (L.) Hook. f. & Thomson.	Bor-ra-petch	MENISPERMIACEAE	Vine	Bitter	Antipyretic, promote the appetite	4

Table 1 Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Thai names	Family names	Used Parts	Taste	Traditional Use	Proportion in Remedy All portion = 211
53	*Trachyspermum ammi* (L.) Sprague ex Turrill	Tien-yao-wa-pha-ni	APIACEAE	Seed	Sweet	Carminative, expectorants, antiemetic, heamatinic	4
54	*Vetiveria zizanioides* (L.) Nash	Phaek-hom	POACEAE	Root	Fragrant	Cardiotonic, carminative, haematinic	4
55	*Zingiber officinale* Roscoe	Khing	ZINGIBERACEAE	Root	Spicy	Carminative, antiemetic	3

Table 2 Analysis of Antibacterial Activities of Yahom-Navakot Remedy's Ingredients

No.	Scientific names	Essential oil		Extract		Reference
		Gram-positive	Gram-negative	Gram-positive	Gram-negative	
1	*Amomum krevanh* Pierre ex Gagnep.	*B. subtilis* *E. coli*				[23]
2	*Anethum graveolens* L.			*S. aureus*	*E. coli* *P. aeruginosa* *S. typhimurium* *S. flexneri*	[24]
3	*Angelica dahurica* Fisch ex Hoffm.			*S. aureus*		[25]
4	*Angelica sinensis* (Oliv.) Diels.			*S. aureus* *S. chromogenes* *S. uberis*		[26]
5	*Artemisia annua* L.			*F. nucleatum* *P. intermedia*		[27]
6	*Atractylodes lancea* Thunb.	*S. aureus* *B. subtilis* *B. cereus*	*E. coli* *P. vulgaris* *P. aeruginosa*			[28]
7	*Brucea javanica* (L.) Merr.				*P. aeruginosa*	[29]
8	*Cinnamomum bejolghota* (Buch.-Ham.) Sweet.	*S. aureus* *B. subtilis* *B. cereus*	*E. coli* *P. aeruginosa* *S. typhimurium*			[30]
9	*Cinnamomum loureirii* Nees.	*L. monocytogene* *S. aureus*	*E. coli* *S. anatum*			[31]
10	*Coriandrum sativum* L.		*E. coli* *S. typhi*			[32]
11	*Cuminum cyminum* L.			*S. aureus* *B. pumilus*	*E. coli* *P. aeruginosa*	[33]
12	*Cyperus rotundus* L.			*S. aureus*	*E. coli*	[34]

Table 2 Analysis of Antibacterial Activities of Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Essential oil		Extract		Reference
		Gram-positive	Gram-negative	Gram-positive	Gram-negative	
13	*Eleusine indica* (L.) Gaertn.			MRSA	*P. aeruginosa* *S. choleraesuis*	[35]
14	*Foeniculum vulgare* Mill.	*S. albus* *B. subtilis*	*S. typhimurium* *S. dysenteriae* *E. coli*			[36]
15	*Glycyrrhiza glabra* L.			*S. aureus* *B. subtilis*	*P. aeruginosa* *E. coli*	[37]
16	*Jasminum sambac* (L.) Aiton.	*S. mutans* *L. casei*	*E. coli*			[38]
17	*Kaempferia galangal* L.			*B. subtilis*	*K. pneumoniae* *P. aeruginosa* *E. aerogenes* *E. coli*	[39]
18	*Lepidium sativum* L.	*S. aureus* *B. subtilis*	*E. coli* *P. aeruginosa* *S. enterica* *K. pneumoniae*			[40]
19	*Ligusticum sinense* Oliv.	*B. subtilis* *S. aureus*	*A. tumefaciens* *E. coli* *P. lachrymans* *X. vesicatoria* *S. haemolyticus*			[41]
20	*Mammeas siamensis* T. Anders.			*S. aureus* *B. subtilis*		[42]
21	*Mesua ferrea* L.			*E. coli* *Vibrio* spp. *S. typhimurium*		[43]
22	*Mimusops elengi* L.			*E. coli* *P. vularis* *K. pneumoniae* *P. aeruginosa*		[44]

Table 2 Analysis of Antibacterial Activities of Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Essential oil		Extract		Reference
		Gram-positive	Gram-negative	Gram-positive	Gram-negative	
23	*Myristica fragrans* Houtt. (Mace)			*S. mutans* *S. mitis* *S. salivarius*	*A. actinomycetemcomitans* *P. gingivalis*	[45]
24	*Myristica fragrans* Houtt. (Nutmeg)			*B. subtilis*	*P. putida* *P. aeruginosa*	[46]
25	*Myristica fragrans* Houtt. (Wood)			*S. aureus* MRSA *S. pyogenes*	*P. aeruginosa*	[47]
26	*Nelumbo nucifera* Gaertn.			*B. subtilis* *S. aureus*	*E. coli* *K. pnemoniae* *P. aeruginosa*	[48]
27	*Nigella sativa* L.	*Staphylococci* spp.				[49]
28	*Phyllanthus emblica* L.			*B. cereus* *S. aureus*	*S. typhi* *E. coli* *S. paratyphi* *Vibrio* spp.	[50]
29	*Picrorhiza kurroa* Royle ex Benth.			*S. aureus* *M. luteus* *B. subtilis*	*P. aeruginosa* *E. coli*	[51]
30	*Pimpinella anisum* L.			*B. cereus* *S. aureus*	*S. typhimurium* *E. coli*	[52]
31	*Piper longum* L.			*S. aureus*	*P. aeruginosa* *V. cholerae*	[53]
32	*Piper ribesioides* Wall.	*B. cereus* *B. subtilis* *S. aureus*	*E. coli* *P. aeruginosa* *K. pneumoniae*			[54]
33	*Piper sarmentosum* Roxb.			MRSA		[55]
34	*Plantago ovata* Forssk.			*B. sphaericus* *B. subtilis*	*P. aeruginosa*	[56]

Table 2 Analysis of Antibacterial Activities of Yahom-Navakot Remedy's Ingredients (Cont.)

No.	Scientific names	Essential oil		Extract		Reference
		Gram-positive	Gram-negative	Gram-positive	Gram-negative	
35	*Plumbago indica* L.			*S. aureus*	*S. typhi* *S. paratyphi*	[57]
36	*Pogostemon cablin* (Blanco) Benth.			*S. aureus* MRSA *S. pyogenes*	*P. aeruginosa*	[58]
37	*Syzygium aromaticum* (L.) Merrill & Perry.		*Serratia* spp. *Salmonella* spp. *Kluyvera* spp. *Klebsiella* spp. *E. coli*			[59]
38	*Terminalia bellirica* (Gaertn.) Roxb.			MRSA	*Acinetobacter* spp. *P. aeruginosa*	[60]
39	*Terminalia chebula* Retz.			*S. aureus* *B. polymyxa* *B. cereus*	*P. aeruginosa* *K. pneumoniae* *S. typhi* *E. coli*	[61]
40	*Tinospora crispa* (L.) Hook. f. & Thomson.			*S. pneumonia* *S. aureus* *C. diphtheria*	*S. flexneri*	[62]
41	*Trachyspermum ammi* (L.) Sprague ex Turrill.			*E. faecalis* *S. aureus*	*E. coli* *K. pneumoniae* *P. aeruginosa* *S. typhi* *S. typhimurium* *S. flexneri*	[63]
42	*Vetiveria zizanioides* (L.) Nash.	*E. faecalis*	*E. cloacae* *E. coli* *P. vulgaris*			[64]
43	*Zingiber officinale* Roscoe.				*S. typhi*	[65]

Table 3 Antibiotic Enhancing Effects of Yahom-Navakot Remedy's Ingredients

No.	Scientific names	Names	Used parts	Antibiotic enhancing effects	References
1	*Carum carvi* L.	Tien-ta-kob	Seed	• Caraway enhanced the bioavailability of cefdinir (89%) and cloxacillin (100%). • The extracts and its fractions enhanced 20 - 110% the bioavailability of antibiotics, anti-fungal, antiviral, and anticancer drug.	[14, 66]
2	*Cuminum cyminum* L.	Tien-khaow	Seed	• The composition contained *C. cyminum* L extracts or the fractions can provide antibiotic bioenhancing activity in the range of 25 - 335%.	[14]
3	*Glycyrrhiza glabra* L.	Cha-em-thet	Root	• Glycyrrhizin; the main chemical component of *Glycyrrhiza glabra* L., enhanced bioactivity of antibiotics such as rifampicin, ampicillin, tetracycline, and nalidixic acids against bacteria strains; for examples, *M. smegmatis, B. subtilis,* and *E. coli*.	[14]
4	*Nigella sativa* L.	Tien-dum	Seed	• Methanol and hexane extracts of *Nigella sativa* L. increased the permeation of amoxicillin significantly ($P < .001$) as compared to the control.	[67]
5	*Pimpinella anisum* L.	Tien-sat-ta-but	Seed	• Aniseeds waste residue extract showed synergistic effect with cephradine against *S. Pneumonia* and *S. aureus* via disc diffusion method.	[68]
6	*Sophora tomentosa* L.	Sa-ra-phat-phit	Seed	• Sophora flavanone B; the main chemical constituent from *Sophora tomentosa* L., markedly reduced the MICs of the β-lactam antibiotics: AMP and oxacillin, aminoglycosides gentamicin, quinolones ciprofloxacin and norfloxacin against *S. aureus;* MRSA.	[69]
7	*Trachyspermum ammi* (L.) Sprague ex Turrill.	Tien-yao-wa-pha-ni	Seed	• The essential oil from *Trachyspermum ammi* L. Sprague ex Turrill reduced the MICs and inhibition zone of gentamicin against *S. aureus;* MRSA.	[70]
8	*Zingiber officinale* Roscoe.	Khing	Rhizome, root	• Ginger enhanced the bioavailability of rifampicin by 65% and ethionamide by 56%. It also enhanced the bioavailability of antibiotics (Azithromycin - 78%) • The composition containing *Z. officinale* alone provided bioavailability activity in the range of 30 - 75%.	[14, 64]

•The composition containing Z. officinale Roscoe. alone provided bioavailability activity in the range of 30 - 75% [14, 64]

Methods

1. Plant Material and Extract Preparation

YN is composed of 55 herbs, shown in Table 1. All YN's ingredients were bought from a local market in Bangkok, Thailand. All 55 crude drugs were identified by a Thai traditional doctor, and then mixed according to the Thai National essential drug list. The herbal mixture was macerated with 95% ethanol for three days. The 95% ethanolic extract of Yahom-Navakot remedy (YNE) was filtered through Whatman no.1 filter paper and concentrated using a rotary evaporator. The crude extract was allowed to dry at 50°C and refrigerated at -20°C. The extract was redissolved by dimethyl sulfoxide (DMSO) before antibacterial testing.

2. Antibiotic Preparation

Thammasat University Hospital, Thailand, supplied levofloxacin used in this study. Levofloxacin was prepared at 100 mg/mL concentration by dissolving levofloxacin 500 mg (Tablet) in distilled water and mixed by vortex mixer until completely dissolved. The dissolved levofloxacin was kept in the refrigerator at 4°C.

3. Bacterial Strain

The pathogenic bacteria were selected based on the incidence rates of antibiotic resistances and common clinical bacterial pathogens. All bacterial strains were purchase from ATCC and DMST. These were *Staphylococcus aureus* (DMST 20651), Methicillin-Resistant *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC BAA 2789), *Salmonella typhi* (DMST 22842), *Shigella dysenteriae* (DMST 15111), and *Escherichia coli* (ATCC 25922). *P. aeruginosa,* MRSA, *S. typhi, S. dysenteriae,* and *E. coli* were antibiotic resistant bacteria strains according to WHO criteria.[72] Selected bacterial strains were cultured in nutrient agar (NA) plates and incubated at 37°C for 24 hours. The cultured bacterial strains were transferred from NA plate to tubes containing Mueller-Hinton broth (MHB) and incubated at 37°C for 2 hours. The turbidity of selected bacterial strain was controlled at 0.5 McFarland before the determination of antibacterial activities.

4. Determination of Minimum Inhibitory Concentration (MIC)

Modified Resazurin in broth microdilution assay[15] was used to determine the minimum inhibitory concentration of YNE and the combination therapy of levofloxacin and YNE. The concentration of levofloxacin was 0.003 - 100 µg/mL and 0.039 - 5 mg/mL for the YN extract. The mixtures were prepared by 2-fold serial dilutions in MHB. The concentrations of YNE were fixed at MIC values against each selected bacterial strains. The final volume was 100 µL, and the final bacterial concentration was 5×10^5 CFU/mL in each well. The controls included, MHB alone, MHB containing selected bacterial strains, and MHB+0.02% DMSO. A 96-well plate was incubated at 37°C for 18 hours. In the evaluation step, resazurin (10 µL) was added into a 96-well plate and incubated again for 2 hours. The resazurin color was observed and recorded for antibacterial activity evaluation. All tests were done in triplicate.

5. Determination of Minimum Bactericidal Concentration (MBC)

After determining MIC values, a portion of solution (5 µL) from each well that showed no growth of bacteria was streaked onto a nutrient agar plate and incubated at 37°C for 24 hours. The minimum concentration with no bacterial growth was determined to be the MBC value.

Results

From the results of antibacterial activities of YNE, the extract showed antibacterial effects against selected bacterial strains including *S. aureus* (MIC = 0.625 mg/mL), MRSA (MIC = 0.625 mg/mL), and *S. epidermidis* (MIC = 0.3125 mg/mL). However, YNE did not have inhibitory activities against other selected bacterial strains (MIC > 5 mg/mL).

In the determination of the combination of levofloxacin and YNE in the antibacterial activities assay, the concentrations of the extract at MIC values against each selected bacterial strains were used (*S. aureus*; MIC = 0.625 mg/mL, MRSA; MIC = 0.625mg/mL, and *S. epidermidis*; MIC = 0.3125 mg/mL). On the other hand, for the non-susceptible bacterial strains the MIC was greater than 5 mg/mL (*K. pneumoniae, P. aeruginosa, E. coli, S. dysenteriae,*

and *S. typhi*). The combination therapy with levofloxacin and YNE showed lower MIC and MBC values against MRSA and *S. epidermidis* than levofloxacin alone. The combination showed the most effective against MRSA as demonstrated by reduced MIC and MBC values (MIC < $3x10^{-8}$ µg/mL and MBC < $3x10^{-8}$µg/mL) compared with levofloxacin alone (MIC = 6.25 µg/mL, MBC = 25 µg/mL). An attempt was made to determine MIC and MBC values by reducing the proportion of YNE by half, however this combination did not show any enhancing effect (Table 4). These findings suggest that the concentration of YNE in the combination was the optimal concentration for a synergistic effect with levofloxacin. For *S. epidermidis*, the MIC value of the combination was equal to levofloxacin's MIC value (MIC = 0.048 µg/mL) but the MBC value was lowered (MBC combination = 25 µg/mL, MBC levofloxacin = 50 µg/mL). The antibacterial activities of the combination against *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, S. dysenteriae,* and *S. typhi,* did not show any enhancing effect of YNE with levofloxacin (Table 4).

Discussion

The combination testing between herbs and antibiotics of YNE had a potent enhancing effect with Levofloxacin against MRSA and *S. epidermidis* by decreasing MIC and MBC. However, it did not affect other selected bacteria strains. It was the first study on the antibiotic enhancing effect of YNE and demonstrated the value of herbal medicine in combination with conventional medicine. There were many studies about antibiotic enhancing effects of natural plants. Cai et al. studied Chinese herbal medicine combined with antibiotics to reduce drug-resistant enterobacteria and non-fermentative bacterial infection.[12] The combinations showed better outcomes than using antibiotics alone.[11] There were many advantages of the combination between herbs and antibiotics such as enhancement of antibacterial activity, treatment of mixed or severe infections, reducing the time needed for long-term antibacterial therapy and prevention of the emergaence of antibiotic resistant bacteria.[71]

All studies above support the use of many natural plants as antibiotic enhancers. It is fascinating for many researchers worldwide to study the combination between natural plants and modern medicines that it could expand the antibacterial spectrum, reduce the emergence of resistant mutants, and decrease toxicity.[16] The previous studies described that there were many antibiotic enhancing mechanisms of herbs such as bacterial active site modification, bacterial receptor blocking,[17] active efflux[18] enzymatic degradation, antibiotic modification,[19] and accumulation of the antibiotic within the bacterial cell due to decreased outer membrane permeability.[20] However, the study about mechanisms is still ongoing to investigate the precise mechanisms.

YN is a well-known Thai remedy and listed in the Thai essential regimens. From the analysis of YN's ingredients in Table 1, all YN's ingredients could be classified into 6 groups by their tastes. The most frequently found taste was spicy (23 herbs) followed by bitter (14 herbs), fragrant (10 herbs), sweet (5 herbs), sour (2 herbs), and astringent (1 herb). Previous studies reported that the biomarkers of some spicy herbs had antibacterial effects.[21] Bitter herbs also showed antibacterial effects and there was a positive correlation between bitter taste and antibacterial effects.[22] These studies supported Thai traditional medicine principles that spicy and bitter herbs enhance the immune system and eliminate pathogens. Moreover, there were 45 from 55 herbs in YN's ingredients that demonstrated antibacterial activities related to the results of YN on antibacterial activities (Table 2).

Most of the YN's ingredients are herbs containing many alkaloids, glycosides, fatty acids, and volatile oils. *P. longum* L., one of YN's ingredients, contains an alkaloid named piperine. The previous studies showed that piperine had an efflux pump inhibited effect and can enhance multiple drugs' activity as well as a nutritional bioenhancer, which enhanced bioavailability and absorption of nutrients by acting on the gastrointestinal tract.[14] There were reports on some of YN's ingredients that had antibiotic enhancing effects shown in Table 3. These were *Carum carvi, Cuminum cyminum, Glycyrrhiza glabra, Nigella sativa, Pimpinella anisum, Sophora tomentosa, Trachyspermum ammi,* and *Zingiber officinale.*

Previous studies on YN's ingredients as antibiotic enhancers, supported our results in combining levofloxacin and YNE which specifically

Table 4 Antibacterial Activities of the Combination of Levofloxacin and Yahom-Navakot Remedy Extract Expressed as MIC Assessed by Modified Resazurin in Broth Microdilution Assay

Sample	KPN700603		PAG9027		ECO25922		SAU25923		MRSA20651		SED12228		SDT15111		STP22842	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
LEV (μg/mL)	0.09	0.19	0.09	0.09	0.006	0.006	0.048	0.39	6.25	25	0.048	50	0.003	0.003	0.003	0.006
YNE (mg/mL)	> 5	-	> 5	-	> 5	-	0.625	1.25	0.625	1.25	0.3125	2.5	> 5	-	> 5	-
LEV + YNE (μg/mL)	0.195	0.19	0.195	0.19	0.006	0.006	0.048	0.39	$< 3x10^{-8}$	$< 3x10^{-8}$	0.048	25	0.003	0.003	0.006	0.006
LEV + 1/2YNE (μg/mL)	-	-	-	-	-	-	0.097	0.39	6.25	> 100	0.097	50	-	-	-	-

Abbreviation: LEV = Levofloxacin, YNE = 95% ethanolic Yahom-Navakot remedy extract, KPN700603 = *Klebsiella pneumoniae* ATCC 700603, PAG9027 = *Pseudomonas aeruginosa* ATCC 9027, ECO25922 = *Escherichia coli* ATCC 25922, SAU25923 = *Staphylococcus aureus* ATCC 25923, MRSA20651 = *Staphylococcus aureus;* MRSA DMST 20651, SED12228 = *Staphylococcus epidermidis* ATCC 12228, SDT15111 = *Shigella dysenteriae* DMST 15111, STP22842 = *Salmonella Typhi* DMST 2284

active against MRSA. Our findings could be applied to reduce the dose, drug-resistant problem, and to increase the efficacy of levofloxacin.

Because of the limitation of this study, the results of antibiotic enhanced effect did not show in fractional inhibitory concentration (FIC) because some of the MIC and MBC values were less than the minimum concentrate of experimental method ($< 3x10^{-8}$µg/mL). The solution was reducing the proportion of YNE by haft and determine MIC and MBC values, however these results did not show any enhancing effect.

In the future, the study about antibiotic enhanced effects of YN in synergistic effect, additive effect, antagonism effect, and time kill study should be considered to understand the mechanism about antibiotic enhanced effects of YN. *In vivo* and clinical studies are essential to evaluate YN's efficacy and safety.

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