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Antimicrobial and antihelmintic activities of asarone rich herbal materials: A review

Trisia Windy, Ika Rahayu and Kris Herawan Timotius

Abstract
Several plants are recognized as asarone rich plants. As a bioactive compound, asarone has benefits is to treat infectious diseases. The aims of this review are to compile information from previous articles on the antifungal and antibacterial activities of asarone rich herbal materials. Several plants are good source for asarone, namely *Acorus calamus*, *Acorus gramineus*, *Eusideroxylon zwageri*, *Perilla frutescens*, *Piper cubeba*, *Pycnocycla spinose* and *Sphallerocarpus gracilis*. Ethyl acetate extracts/fractions have good antifungal activity followed by essential oil. *Candida albicans* and many filamentous fungi can be inhibited by ethyl aceate extract and essential oils from asarone rich herbal materials. Only essential oils, not ethyl acetate extract, are good in inhibition either Gram negative or Gram positive bacteria. Isolated asarone also shows antimicrobial activity. The asarone content in the extract depends on the polarity of the solvent. The nonpolar extract trends to have higher asarone than the polar. It can be concluded that the essential oils from asarone rich herbal material have good inhibitory activity against pathogenic yeast/fungal and bacteria. The ethyl acetate extract is only effective in inhibition of pathogenic yeast or filamentous fungi.

Keywords: *Acorus calamus, Eusideroxylon zwageri, Piper cubeba, Candida albicans*

1. Introduction
Several plants are known as asarone rich herbal materials, for example, *Acorus calamus* [1, 2], *Acorus gramineus* [3], *Piper cubeba*, *Sphallerocarpus gracilis* [4], *Perilla frutescens* [5], and *Eusideroxylon zwageri* [6] (Table 1). In the case of *A. calamus*, it is traditionally used to treat several diseases, such as diabetes [7], Alzheimer [8], bacterial pneumonia, obstructive emfisema, lung inflammation, acute and chronis bronchitis, bronchial ashma, etc. [4].In the case of *Acorus gramineus*, its extracts or active components have been used in traditional medicine for cognition impairment and neuroprotection [9], mainly for sedation and enhancement of brain function. *A. gramineus* possesses anti-allergic activity [10] and has been used to treat senile dementia, stroke, and cardiovascular disease [11] *A. gramineus* is often used to treat arthritis by reducing the inflammation indicators [12], and to treat an anxiolytic-like activity [13]. Antifungal activities from the extract of *A. gramineus* is also reported [3]
Many reports on extract's antimicrobial activity from *A. calamus* [14] and *A. gramineus* are found. The essential oil of *A. calamus* and its major compound β-asarone have antimicrobial against Gram-positive, Gram-negative bacteria, and fungi [15]. Alcoholic rhizome extracts of *A. calamus* are found to have anthelmintic and antibacterial activity [16], and anti-Candida [17].
This review aims to compile and evaluate scientific information available on the antimicrobial activity of asarone rich herbal materials, mainly their antifungal and antibacterial activities, antihelmintic and antivirus. The delivery of asarone rich herbal extract is then discussed.

Table 1: Asarone rich herbal material (ARHM)

Species	Preparation	Asarone	Ref.
Acorus calamus	Leave, rhizome water or MeOH extract	Asarone (70 – 96%)	[1, 2]
Acorus gramineus	Rhizome MeOH extract	Asarone (80%)	[3]
Acorus gramineus	Rhizome essential oil	α-asarone (63-81%) R-asarone (8-14%)	[3]
Sphallerocarpus gracilis	Seed essential oil	a-asarone (33.12%)	[4, 18]
Perilla frutescens	Leave, seed, stem MeOH extract	α-asarone (11.85%)	[19, 5]
Pycnocycla spinosa	Aerial part essential oil	cis-Asarone (62.5%)	[20]
Eusideroxylon zwageri	Ethanol seed extract	Asarone 80%	[6]

2. Antifungal activity of asarone rich herbal material

As seen in the Table 2, various extracts of asarone rich herbal materials (ARHM) have inhibitory activities against human yeasts and molds. Among them, two human important yeast/fungi are inhibited by ARHM. They are *Candida albicans* and filamentous fungi belong to dermatophytes, such as *Trichophyton rubrum* and *Microsporum gypseum*. It is clearly suggested that extracts or ARHM possess α- and β-asarones that are responsible for their antiyeast and antifungal activities.

The most active extract is ethyl acetate extract, followed by essential oils. These nonpolar extracts contain more asarone than the polar extract. Several experiments show that isolated asarone can inhibit the growth of pathogenic yeast and filamentous fungi. Anti-Candida potential of *A. calamus* extract is determined by the presence of bioactive compound, β-asarone. Isolated asarone is highly toxic to Candida [21]. β-Asarone is able to make changes in *C. albicans* cell morphology and biofilm formation. The inhibitory ability of β-asarone is maybe at the ergosterol biosynthesis. β-asarone is so far considered as non-toxic and therefore safe as a topical antifungal agent. [21] Unfortunately, there is no antifungal activity is observed from water extract.

The antifungal activity of β –asarone is better thatα-asarone. Inhibition zone (20-29 mm) and MIC of β-asarone (0.5-8 mg/ml) are better than inhibition zone (19-27 mm) and MIC of α-asarone (2-8 mg/ml) [22]. Under scanning electron microscopic observation, both asarones treated hyphae and conidia are shrunken and collapsed, that indicates the cell fluid leakage [2]. The rhizome extract, especially ethyl acetate extract, has not only good antimicrobial activity but inhibitory adhesion and biofilms formation. [17]

Table 2: list of asarone-inhibited yeast and filamentous fungi

Species	ARHM	Part	Extract	ZI mm	MIC mg/ml	MFC mg/ml	Ref.
Candida albicans	*A. calamus*	R	Hexane	ND	20.0	ND	[21]
			EtAc	25.0	4.0	ND	[22]
			EtAc	23.0	4.0	ND	[22]
			MeOH	9.3	0.1	0.3	[2]
			EtOH	ND	5.0	ND	[23]
			Asarone	ND	8.0	ND	[21]
		L	EtAc	23.0	8.0	ND	[22]
	Pycnocycle spinosa	S	EO	10.2	1.0	2.0	[20]
		Ap	EO	9.5	1.0	4.0	[20]
	P. fabellifolia	Ap	EO	6.9	1.0	4.0	[20]
Cryptococcus neoformans	*A. calamus*	R	β-asarone	24.8	0.5	0.5	[2]
Cryptococcus gastricus	*A. calamus*	R	EtAc	22.0	5.0	ND	[22]
		L	EtAc	20.0	6.0	ND	[22]
Saccharomyces cerevisiae	*A. calamus*	R	β-asarone	10.0	1.0	1.0	[2]
Aspergillus niger	*A. calamus*	R	EtAc	25.0	2.0	ND	[22]
			EO	ND	ND	0.1	[15]
			EtOH: EtAc frc	20.0	ND	ND	[24]
			EO	21.5	ND	ND	[25]
			β-asarone	ND	ND	0.4	[15]
		L,R	EO	ND	2.0	ND	[26]
	Pycnocycle spinosa	S	EO	6.8	0.3	0.3	[20]
		Ap		6.8	0.5	0.5	[20]
	P. flabellifolia	Ap	EO	6.8	0.5	0.5	[20]
Aspergillus flavus	*A. calamus*	R	EO	18.5	ND	ND	[25]
Penicillium chrysogenum	*A. calamus*	L	EtAc	20.0	3.2	ND	[22]
		R	EtAc	22.0	3.2	ND	[22]
			EO	ND	ND	0.2	[15]
			β-asarone	ND	ND	1.2	[15]
Trichophyton rubrum	*A.calamus*	R	MeOH	IC50: 0.2 mg/ml			[2]
Microsporum gypseum	*A. calamus*	R	MeOH	IC50: 0.2 mg/ml			[2]

Note: ARHM: asarone rich herbal materials; Ap: aerial part; L: leave; NA: no activity; ND: not determined; NZ: no inhibition zone; R: rhizome; S: seed, EtAc: ethyl acetate; MeOH: methanol; EO: essential oil.

3. Antibacterial activity of asarone rich herbal material

A list of tested bacteria can be seen in Table 3 and Table 4, for Gram-negative and positive bacteria, respectively. The essential oil from *A.calamus* that contains asarone in high percentage can inhibit Gram- negative and Gram-positive bacteria. Essential oils of ARHM can inhibit many species of Gram-negative bacteria, such as *Escherichia coli*, Klebsiella, Pseudomonas, and Salmonella. But other extracts, semi and polar extracts can only inhibit *Escherichia coli*, and not effectively inhibit other Gram-negative bacteria. There is no antibacterial activity from water extract (Table 3). Essential oil is also very effective in the growth inhibition of Gram-positive bacteria. The growth of various species of Bacillus, and *Staphylococcus aureus*, including MRSA can be inhibited by the essential oil of ARHM. In many cases, ethyl acetate extract is not effectively able to inhibit the growth of bacteria. Hexane or petroleum ether extracts are able to inhibit many bacteria, even less effective compared with essential oils.

Isolated *β-asarone* at very low concentration (0.05%) is able to inhibit *E. coli* and *S. aureus*. This inhibitory capacity is better than the essential oil of ARHM, and essential oils from other plants, such as essential oil from ginger (concentration 25%) and ylang-ylang (concentration 3, 1%) [27]. Ethyl acetate and ethanol rhizome extracts exhibit pronounced antibacterial activity against MRSA with diameter zone of inhibition (22-26mm). [24] This essential oil can not inhibit other bacteria, such as *Salmonella parathypi* and *Shigella sonnei*, *Enterococcus faecalis* [14]. Rhizome and leave extracts from *A.*

calamus possess α- and β-asarones that are responsible for their antimicrobial activities [22].

The inhibitory mechanism of extracts from ARHM is probably different for fungal inhibition and for bacterial inhibition. Under scanning electron microscopy, there is no change (shrunken or collapsed) in the bacterial cell morphology as observed in the yeast or filamentous fungi, after treatment with methanol extract of *A. calamus* that contains β-asarone. β-asarone bioactive compound does not disrupt the cell wall and cell membrane of the bacterial cell [2]. But, SEM observations showed that essential oil from *S. gracilis* can damage the physical and morphological alteration to microorganisms [4]. Also, scanning electron microscopy study of the ethanol extract of *P. frutescens* reveals potential detrimental effects on the morphology of *P. aeruginosa* [28] and *S. aureus* [29].

Table 3: List of tested Gram negative bacteria

Bacteria	ARHM	Part	Extract	IZ mm	MIC mg/ml	MBC mg/ml	Ref.
Enterobacter aerogenes	*A. calamus*	R	EO	ND	ND	0.6	[15]
			β-asarone	ND	ND	NA	[15]
Escherichia coli	*A. calamus*	R	hexane	7.2			[14]
				NA	ND	ND	[30]
				3.0	3.9	7.8	[31]
			PE	13.5	0.25	ND	[32]
			Chl.	NA	ND	ND	[30]
				3.3	3.9	7.8	[31]
			EtAc	25.0	42.0	ND	[22]
			EtAc	20	16	ND	[22]
			EO	ND	ND	1.1	[15]
			EO	6.7	4.0	ND	[33]
			MeOH	8.5	ND	ND	[14]
			EtOH	3.1	3.9	7.8	[31]
			Water	NZ	ND	ND	[14]
			Water	NZ	ND	ND	[30]
			β-Asarone	6.8	10	>10	[2]
				ND	ND	NA	[15]
		L	hexane	9.0	ND	ND	[14]
			EtAc	22.0	42.0	ND	[22]
				18	18	ND	[22]
			MeOH	8.8	ND	ND	[14]
			Water	NZ	ND	ND	[14]
		Soap	EO	11.7	ND	ND	[27]
	Pycnocycla spinosa	S	EO	6.8	4.0	8.0	[20]
		Ap	EO	6.8	4.0	8.0	[20]
	Pycnocycla flabellifolia	Ap	EO	13.1	2.0	4.0	[20]
	S. gracilis	S	EO	18.5	160.0	320.0	[4]
Klebsiella pneumonia	*A. calamus*	R	EO	ND	ND	0.5	[15]
			βasarone	ND	ND	1.0	[15]
	S. gracilis	S	EO	26.4	80.0	160.0	[4]
Proteus vulgaris	*A. calamus*	R	EO	ND	ND	0.7	[15]
			βasarone	ND	ND	0.5	[15]
Proteus mirabilis	*A, calamus*	R	EO	ND	ND	0.5	[15]
			βasarone	ND	ND	0.5	[15]

Note: ARHM: asarone rich herbal materials;Ap: aerial part; L: leave; NA: no activity; ND: not determined; NZ: no inhibition zone; R: rhizome; S: seed; EtAc: ethyl acetate; MeOH: methanol; EO: essential oil; Chl.: chloroform; PE: petroleum eter; EtOH: ethanol

Table 3: List of tested Gram-negative bacteria (Cont.)

Bacteria	ARHM	Part	Extract	IZ mm	MIC mg/ml	MBC mg/ml	Ref.
P.aeroginosa	*A.calamus*	R	EtAc	NZ	ND	ND	[22]
			EO	ND	ND	1.7	[15]
			β -asarone	ND	ND	NA	[15]
			MeOH: EtAc fr	NZ	ND	ND	[2]
			PE	16.2	0.3	ND	[32]
		L	EtAc	NZ	ND	ND	[22]
	Pycnocycla spinosa	S	EO	6.8	4.0	4.0	[20]
		Ap		6.8	4.0	8.0	[20]
	Pycnocycla flabellifolia	Ap	EO	7.2	4.0	4.0	[20]
Salmonella typymurium	*A. calamus*	R	EO	ND	ND	1.5	[15]
			β-asarone	ND	ND	NA	[15]
S.typhimurium	*S. gracilis*	S	EO	11.7	320.0	320.0	[4]
Salmonella parathyphi	*A. calamus*	R	EtAc	NZ	ND	ND	[22]
	A. calamus	L	EtAc	NZ	ND	ND	[22]
S.enteritidis	*S. gracilis*	S	EO	14.9	320.0	640.0	[4]
S.typhi	*A. calamus*	L	MeOH	9.3	ND	ND	[14]
			Hexane	8.0	ND	ND	[14]

			Water	NZ	ND	ND	[14]
		R	MeOH	9.9	ND	ND	[14]
			Hexane	8.9	ND	ND	[14]
			Water	NZ	ND	ND	[14]
Serratia marcescens	*A. calamus*	R	EO	ND	ND	1.0	[15]
Shigella sonnei	*A. calamus*	R	EtAc	NZ	ND	ND	[22]
	A. calamus	L		NZ	ND	ND	[22]
Vibrio cholera	*A. calamus*	R	EtAc	NZ	ND	ND	[22]
	A. calamus	L		NZ	ND	ND	[22]

Note: ARHM: asarone rich herbal materials; Ap: aerial part; L: leave; NA:no activity; ND: not determined; NZ: no inhibition zone R: rhizome; S: seed; EtAc: ethyl acetate; MeOH: methanol; EO: essential oil; Chl.: chloroform; PE: petroleum eter; EtOH: ethanol

Table 4: List of tested Gram-positive bacteria

Bacteria	Plant	Part	Extract	IZ mm	MIC mg/ml	MBC mg/ml	Ref.
Bacillus cereus	*A. calamus*	R	Hexane	8.5	ND	ND	[14]
			MeOH	9.0	ND	ND	[14]
			Water	NZ	ND	ND	[14]
		L	Hexane	8.4	ND	ND	[14]
			MeOH	8.5	ND	ND	[14]
			Water	NZ	ND	ND	[14]
	S. gracilis	S	EO	16.1	320.0	640.0	[4]
B.coagulans	*S. gracilis*	S	EO	8.30	640.0	1280.0	[4]
B.megaterium	*Piper cubeba*		MeOH: β-asarone, and asaronaldehyde	7.2 to 9.6	6.3 to 12.5	25.0 to 50.0	[34]
	S. gracilis	S	EO	19.20	160	640	[4]
B.pumilis	*A. calamus*	R	Hexane	-			[30]
			Chl.	-			[30]
			Water	9			[30]
	Piper cubeba		MeOH: β-asarone, and asaronaldehyde	7.2 to 9.6	6.3 to 12.5	25.0 to 50.0	[34]
Bacillus subtilis	*A. calamus*	R	PE	10,4	0.50	ND	[32]
			EO	ND	ND	0.234	[15]
			β -asarone	ND	ND	4.166	[15]
	Piper cubeba		MeOH: β-asarone, and asaronaldehyde	7.2 to 9.6	6.3 to 12.5	25.0 to 50.0	[34]
	S. gracilis	S	EO	20.2	160	320	[4]
Enterococcus faecalis	*Perilla frutescens*		EO		0.5		[35]
	A. calamus	R	EtAc	NZ	ND	ND	[22]
		L	EtAc	NZ	ND	ND	[22]
Enterococcus	*A. calamus*	R	MeOH → EtAc	NZ	ND	ND	[2]
Micrococcus flavus	*A. calamus*	R	EO	ND	ND	0.143	[15]
			β-asarone	ND	ND	4.6	[15]
Micrococcus luteus	*A. calamus*	R	EO	ND	ND	0.032	[15]
			β-asarone	ND	ND	4.6	[15]
	S. gracilis	S	EO	9.3	640	640	[4]
Staphylococcus aureus	*A. calamus*	R	PE	16.2	0.3	ND	[32]
			Hexane	5.3	3.9	7.8	[31]
			Hexane	12.0	ND	ND	[14]
			EtAc	NZ	ND	ND	[22]
			MeOH → EtAc	9.2	5	>10	[2]
			chloroform	7.3	3.9	7.8	[31]
			EO	8.8	4.0	ND	[33]
			EO	ND	ND	0.6	[15]
			MeOH	12.0			[14]
			EtOH	5.9	3.9	7.8	[31]
			Water	NA			[14]
			β-asarone	ND	ND	NA	[15]
		L	Hexane	7.8	ND	ND	[14]
			EtAc	NZ	ND	ND	[22]
			MeOH	12.0	ND	ND	[14]
			Water	NA	ND	ND	[14]
		soap	Essential oil	15.5	ND	ND	[27]
	Pycnocycla spinosa	S		10.2	1.0	2.0	[20]
		Ap		9.5	1.0	1.0	[20]
	Pycnocycla flabellifolia	Ap		6.9	1.0	4.0	[20]
	Perilla frutenscens	L	EtOH				[29]

	S. gracilis	S	EO	22.40	160	320	[4]
MRSA	*A. calamus*	R	MeOH → EtAc	10.8	5	>10	[2]
MRSA	*A.calamus*	R	EtOH → EtAc	22-26			[24]
S.epidermidis	*S. gracilis*	S	Essential oil	10.90	640	640	[4]
	A. calamus	R	Essential oil	ND	ND	0.520	[15]
		R	β-asarone	ND	ND	NA	[15]

Note: ARHM: asarone rich herbal materials; Ap: aerial part; L: leave; NA: no activity; ND: not determined; NZ: no inhibition zone; R: rhizome; S: seed; EtAc: ethyl acetate; MeOH: methanol; EO: essential oil; Chl.: chloroform; PE: petroleum eter; EtOH: ethanol

4. Antihelmintic and antivirus of asarone rich herbal material

Interestingly that rhizome extract of *A.calamus* has antihelmintic and antivirus activities. Not much research is carried out for these potentials. The rhizomes of *Acorus calamus* are used as anthelmintics. The isolated β-asarone has stronger antihelmintic activity [16]. The extracts of *A. calamus* and *A.gramineus*are able to exhibit antihelmintic activity [16]. The rhizomes of *A. calamus* have been traditionally used to cure intestinal-helminthic infections. The β-asarone has slightly better anthelmintic effects than crude extract. *A. calamus* is containing high β-asarone that has a great potential to be used as anthelmintic herbal products [36].

Extract of *A. calamus* is reported as possessing anti-dengue activity and anti HIV/AIDS. The methanol and ethanol extracts of *A. calamus*show inhibition of DENV-2 dengue virus serotype 2 (DENV-2). Their extracts influence the viral RNA replication (the early stage), which in turn inhibit mRNA and protein levels of DENV2. These extracts are good candidates for a novel natural DENV inhibitor and a potential candidate for the treatment of DENV infectious disease [37]. *In silico* analisis confirms that the methanol extracts of *A. calamus*show anti-dengue activities, and determines that the free energy of binding between NS5 of dengue virus with bioactive compounds in *A. calamus* extract. Therefore, future research needs to be conducted to find the efficacy of the pure or partially isolated bioactive compounds that can be accepted as a new candidate of antiviral drug [38]. There is evidence of ARHM extracts for the treatment of HIV/AIDS [39] probably due to its inhibitory activity of HIV-1 reverse transcriptase (HIV-1 RT) [40].

5. Delivery of asarone rich herbal material

Two ARHM, *A. calamus* and *A.gramineus* have a long history in traditional medicine, and offers many health benefits. They can be administrated as oral drug, injected drug, intravenous lipid emulsion, and as external or topical drug. But due to their toxicity [1, 41] and anaphylactic reactions [42], they are recommended just for external use as topical ointment [43], nasal administration [44], as added ingredient of transparent soap [45], and forehead administration [46, 47].

Extract ointment can be prepared for wound healing. The activity of ethanolic extracts of *A.calamus* leaves have therapeutic benefits in wound healing [45]. In traditional herbal medicine, *A. calamus* has been used as a wound-healing agent. Their antimicrobial activities are important for the wound healing in order to prevent the growth of the infectious pathogens. The ethanolic extract of *A. calamus* leaves has potential wound-healing activity that is applied topically [45]. Together with its anti-inflammatory activity, the wound-healing effect is also found in aqueous extracts of the fresh roots and rhizomes of *A. calamus*. The most common extracts are aqueous extract or rhizome and ethanol extract of dried leave. Aqueous extracts can be administered topically and can enhance the rate of skin wound-healing significantly [48], even has no antimicrobial activity as discussed before. As β-asarone is lipophilic, it was made into water-soluble formulation by encapsulating into beta methyl cyclodextrin [1]. Nasal administration of asarone is a possible way for drug delivery of ARHM extracts. A drug carrier material can be prepared for nasal administration. This provides the foundation for nasal drug delivery *in vivo* pharmacokinetic study. Many drugs are known for their inability to reach the brain through routine pathways due to the blood-brain-barrier (BBB). Nasal administrations are reported to have a potential direct route to transport drug into the brain avoiding the BBB. They have high bioavailability for treating brain diseases, including infectious diseases in the brain. The further drug development of natural agents from herbal medicines via NBDD (Nose to brain drug delivery) is needed [44].

It is a good idea to add essential oil in the soap. This soap is a good approach for external use of ARHM. It is proved that transparent soap with 0,05-0,2 mL/100 mL essential oil can inhibit the growth of *S.aureus* and *E. coli* with inhibition zone of 15,46 mm and 11,67 mm, respectively [27].

6. Conclusions and Recommendations

6.1 Conclusions

Several plants are rich with asarone. Most of them have valuable role as traditional herbal medicine, except for *Eusideroxylon zwageri* seed that is the least researched ARHM. Antifungal and antibacterial activities of ARHM extract are associated with the asarone content of the extracts**.** Essential oils from ARHM are the most active in inhibiting pathogenic microorganisms, for example *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*. Ethyl acetate extracts are good in inhibition of anti-yeast/fungal and *E. coli*. Hexane and petroleum ether extracts have good inhibitory activity against Gram-negative and positive bacteria. Topical administration of ARHM is suggested rather than the oral administration. Asarone containing ointment, nasal inhaler, and soap are good alternative preparation.

6.2 Recommendations

Further studies of the unexplored ARHM are needed, especially the seed of *Eusideroxylon zwageri*. Asarone as bioactive compounds are mainly found in ethyl acetate extracts and essential oils. In the future, the application of encapsulation and nanotechnology can be tried to enhance the efficacy of the asarone containing drugs. A comprehensive study on stability, safety and efficacy of antifungal and antibacterial bioactive compounds from ARHM need to be further investigated. New approaches, such as nasal and forehead administration and the use of therapeutic soap, also need further investigations because of its indication of great potential for clinical application in the future.

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