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Jalan Ir. H. Juanda No. 20, Bogor 16122
Phone: +62 251 8321746, Faks.: +62 251 8326561
email: iaardpress@litbang.deptan.go.id

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Lampiran 8.

SYSTEMATIC REVIEW AND META-ANALYSIS OF ANTIFUNGAL PROPERTIES OF *Piper betle* Linn

Forman Erwin Siagian

Department of Parasitology, Faculty of Medicine-the Christian University of Indonesia
J. Mayjen Sutoyo No 2. Jakarta
formanerwin@yahoo.com

ABSTRACT

Piper betle Linn, a member of Piperaceae family, is considered as well-known traditional herb plants widely used as traditional medicine/remedies, especially in Asia region. Previous studies reported that it showed various biological activities, both *in vitro* and *in vivo*. This study evaluated antifungal properties of *P. betle* extract and/or chemical constituents, by conducting a systematic review and meta-analysis of related articles published before January 2013. Based on a number of selected criteria, the articles were then carefully examined for quality assessment and data analyses. Twenty five published studies identified were eligible for further analyses and comparisons. The majority of the studies showed that *P. betle* Linn. had promising antifungal properties against several types of fungi; most of them are opportunistic fungi. It is suggested that method of extraction and route of administration determine antifungal activity of *P. betle*.

Keywords: *Piper betle*, plant extract, Betel oil, antifungal activity

INTRODUCTION

Fungal infection emerges annually and has caused major costs because of its potential effect on morbidity and mortality (for human, animal or plant) and its ability to cause food spoilage that lowers quality and acceptability of food products[1-30]. Nowadays, synthetic fungicides/antifungals are widely used as primary means to control fungal infection. However, synthetic fungicides are getting more expensive and their uses may lead to increased resistance of fungi against standard antifungals (Kumar *et al.*, 2010; Trakranrungsie, 2011; Chahal *et al.*, 2011). Plant derived products that have antifungal activity/properties become important biocontrols since they are safer, more tolerable, environmentally friendly and widely accepted (Trakranrungsie, 2011). *Piper betle* Linn. is one of the traditional herb plants that meet the criteria (Table 1).

Previous studies have revealed its array of pharmacological activities including oral hygiene, anti-diabetic, cardiovascular, anti-inflammatory/immunomodulatory, anti-ulcer, hepatoprotective and anti-infective. Betel vine (*P. betle* Linn.), a shade-loving, perennial evergreen climber of tropical origin has different names in different regions in Asia. It is estimated that *P. betle* is consumed daily by nearly 600 million people over different regions from east Africa to Polynesia. Betel chewing is a popular habit among native people of those regions. *P. betle* is known for its ethno-medicinal properties and widely used as traditional folk remedies in India, Indonesia, and other Indo-China regions-Malaysia, Vietnam, Laos, Cambodia, Thailand, Myanmar, Singapore and also the far east Asia (Kumar *et al.*, 2010; Trakranrungsie, 2011). This study evaluated antifungal properties of *P. betle* extract and/or chemical constituents, by conducting a systematic review and meta-analysis of related articles published before January 2013.

MATERIAL AND METHOD

Literature search was conducted through Yahoo or Google search engine. The medical subject heading (MeSH) term used as keyword was antifungal properties/activity of *Piper Betle* Linn. Selection criteria for this study was based on methods of extraction, active compounds (if any), fungal species, length

and method of incubation, diameter of inhibition zones or mean inhibitory concentration, *in vitro* or
(Figure 1).

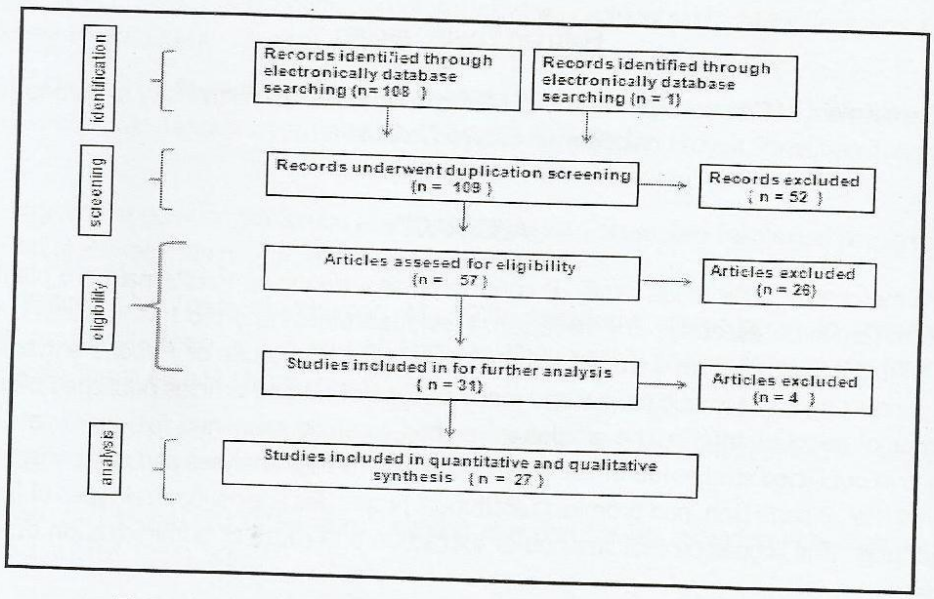

Figure 1. Literature search and selection of published reports

RESULT AND DISCUSSION

We identified hundreds of studies that reported antifungal activity/property of *P. betle* from online databases and reference lists of systematic reviews. It was found that 25 studies met the criteria. There was substantial variability in the published studies examined in terms of plant materials used, methods, organisms tested and antifungal activities. All of these parameters were examined carefully and presented in Table 1.

Table 1. Published studies examined for further analysis.

Study	Plant materials	Methods	Organism tested	Susceptibility
Caburian *and Osi*, 2010	mature leaves	steam distillation	*Candida albicans*	+ MIC250µg/mL
Sharma *et al.*, 2011	leaves and tender roots	extracted in chloroform and methanol extractor (separatedly) in room temperature	*Trichophyton mentagrophytes* dermatophytes:	+ MIC1.95µg/mL
			Trichophyton mentagrophytes MTCC 8476	+ MIC312.5µg/mL
			Trichophyton rubrum MTCC 8477	+ MIC625µg/mL
			Trichophyton tonsurans MTCC 8475	+ MIC312.5µg/mL
			Microsporum gypseum MTCC 8469	+ MIC625µg/mL
Pratiwi, 2009	essentials oils	water extracts	*Pityrosporum ovale*	inhibition zone 16.90Â±2.27mm
Suprapta *and Khalimi*, 2012	leaf, fruit, flower, seed or rhizome	methanol extraction	plant pathogen: *Fusarium oxysporum* f. sp. *Capsici* isolates LS05, LS14, BS01, BS07	Ø inhibition zone ranging 13-21mm
Himratul-Aznita *et al.*, 2011	fresh leaves	crude aqueous extract	human oral Candida species:	
			Candida albicans ATCC 14053	+MIC3.13-100 (~ 12.5) µg/mL
			Candida glabrata ATCC 90030	+MIC3.13-100 (~ 12.5) µg/mL
			Candida tropicalis ATCC 13803	+MIC3.13-100 (~ 12.5) µg/mL
			Candida krusei ATCC 14243	+MIC3.13-100 (~ 12.5) µg/mL
			Candida lusitaniae ATCC 64125	+MIC3.13-100 (~ 12.5) µg/mL
			Candida parapsilosis ATCC 22019	+MIC3.13-100 (~ 12.5) µg/mL
			Candida dubliniensis ATCC MYA-2975	+MIC3.13-100 (~ 12.5) µg/mL

Table 1. Continued ...

Study	Plant materials	Methods	Organism tested	Susceptibility
Ali *et al.*, 2010	leaves→ active ingredients hydroxychavicol	chloroform and aqueous leaf extract	selected fungi: *Candida albicans* ATCC 90028, ATCC 10231 *Candida glabrata* ATCC 90030 *candida krusei* ATCC 6258 *Candida parapsilosis* ATCC 22019 *Candida tropicalis* ATCC 750 *Cryptococcus neoformans* ATCC 204092 *Aspergillus flavus* MTCC 1973, MTCC 2799 *Aspergillus fumigatus* MTCC 1811 *Aspergillus niger* ATCC 16046 *Aspergillus parasiticus* MTCC 2796 *Epidermophyton floccosum* MTCC 613 *Microsporum gypseum* MTCC 2819 *Microsporum canis* MTCC 2820 *Trichophyton mentagrophytes* ATCC 0533 *Trichophyton rubrum* MTCC 296	 + MIC 125-500µg/mL + MIC 15.62-31.25µg/mL + MIC 15.62-31.25µg/mL + MIC 31.25-62.5µg/mL +MIC 125-500µg/mL + MIC 62.5µg/mL + MIC 125-500µg/mL + MIC 125-500µg/mL + MIC 125-250µg/mL +MIC 250µg/mL +MIC 15.62µg/mL +MIC 15.62-31.25µg/mL +MIC 7.81-15.62µg/mL +MIC 15.62-31.25µg/mL +MIC 15.62-62.5µg/mL
Widodo *and Sukmawati*, 2010	leaf	ethanol extraction	*Candida albicans* ATCC 10231	significant reduced number of colonies on application of 2,5%, 5% and 10% cream from day 4^{th} to 10^{th}
Prince *et al.*, 2011	Fresh leaves, Sterilized→ dried	chloroform (c), ethanol (e) and aqueous (a) extract	plant pathogenic fungus, red rot disease causing agent *Colletotrichum falcatum*	c: zone of inhibition 19 mm e: zone of inhibition 25 mm a: zone of inhibition – mm
Trakranrungsie *et al.*, 2006	fresh plant sample	crude ethanolic extract	zoonotic dermatophytes causing skin infection (ringworm) *Microsporum gypseum* *Microsporum canis* *Trichophyton mentagrophytes*	 Ø inhibition zone 20.30±0.29mm Ø inhibition zone 28.00±0.12mm Ø inhibition zone 32.00±0.15mm
Astuti *et al.*, 2010	fresh leaf	ethanolic extract	*Candida albicans*	Anti fungi activity was the same as control positive (mikonazol ointment)
Muniruzzaman and Chowdhury, 2006	fresh leaf/ bulbs		fish pathogenic fungal species: *Aphanomyces invadans* *Saprolegnia* sp *Achlya* sp	 no inhibitory effect no inhibitory effect no inhibitory effect
Reveny, 2011	betel leaf powder	ethanol (e), ethyl acetate (ea), n-hexana (nh) and aqueous (a) extract	*Candida albicans*	e: KHM 10% (9.6mm) ea: KHM 2,5% (11.4mm) nh: KHM 10% (9.3mm) a: negatif
Prakash *et al.*, 2010	essential oil → eugenol, acetyleugenol		*Aspergillus flavus*	+ MIC 0.7 µg/mL
Nuryati and Rahman, 2005	leaf extract	scalded leaf extract	*Aphanomyces* sp	+ MIC 0.1 g/L
Herawati, 2009	leaf extract	ethyl acetate (ea) and n-hexane (nh) extract	*Candida albicans*	ea: decreased number of colony nh: decreased number of colony ea is better antifungal than nh
Dissanayake and Kumari, 2012	leaf extract	methanol extract	wilt of p *Polyscial balfouriana* var *marginata* *Fusarium oxysporum*	 not significant fungicidal effect

Table 1. Continued ...

Study	Plant materials	Methods	Organism tested	Susceptibility
Johnny *et al*, 2010	leaf	methanol (m), chloroform (c) and acetone (a) extraction	plant pathogenic fungus *Colletotrichum gloeosporioides*	+ MIC 17.50-20.00 µg/mL
Lopez *et al.*, 2003	fresh (f) and dried (d) leaf	crude ethanol extract	*Saccharomyces cerevisae*	f:- ; d:+ antimicrobial activity
Mani and Boominathan, 2011	plant material	water, ethanol, methanol, acetone, hexane, butanol extract	*Aspergillus niger* human pathogenic fungus from chronic dental disease affected patients *Candida albicans*	f:- ; d:+ antimicrobial activity ethanol extract showed highest zone of inhibition (mm) while butanol and hexane showed the smallest zone of inhibition
Suprapta and Khalimi, 2009	plant parts	aqueous extract	fungus causing stem rot disease on Vanilla seedlings *Fusarium oxysporum* f.sp. *vanilae*	suppression of radial fungal growth up to 92.4%
Jenie *et al.*, 2001	leaves	Cold and hot water, ethanol and combination of distillation and ethanol extraction	Foodborne pathogens and food spoilage microorganisms: *Aspergillus niger* *Penicillium ruhrum* *Candida utilis* *Saccharomyces cerevisae*	The whole extract (mixture of volatile and non volatile extract) showed the strongest antimicrobial activity (0.025% v/v MIC) towards all Foodborne pathogens and food spoilage microorganisms
Sim *et al.*, 2013	Fresh leaves	Volatile oil in combination with *Myristica fragrans*	*Aspergiilus flavus*	*P. betle* Ø inhibition zone 38mm at 50mg/mL (36% inhibition) *M. fragrans* Ø inhibition zone 46mm at 50mg/mL (26% inhibition) In combination Ø inhibition zone 21mm at 50mg/mL (65% inhibition)
Sathpaty *et al.*, 2011	leaf	Oil extraction	*C albicans* *A. niger*	MIC 30 µl/ml, Ø inhibition zone 24 ±0,57 mm MIC 35 µl/ml Ø inhibition zone 23 ±0,4 mm
Nair and Chanda, 2008	leaves	Aqueous and Methanol extract	*C tropicalis ATCC 4563*	Only methanol extract showed anticanddidal activity
Row and Ho, 2009		Methanolic and aqueous extract	*C albicans* *M. pachydermatis*	Strong activity against both fungus

Most of the published studies used fresh leaves, while some studies used dried leaves except only Suprapta *et al.* (2012; 2009) that used all parts of the plant including leaf, fruit, flower, seed and/or rhizome. Various methods were conducted to extract the active components. The methods ranged from traditional to high end sophisticated instrumental methods. Formulation was also an important issue. Some studies showed antifungal properties of *P. betle* against cutaneous/superficial fungi in the form of gel or shampoo. Principally, this procedure is aimed obtaining pure active ingredients, even though not all of these studies characterized and identified the chemical composition and its antifungal/pharmacological properties. Combination with other substances that have antifungal properties is also beneficial to some extent. A study conducted by Sim *et al.* (2013) showed that mixture of *Piper betel* oil and *M. fragrans* gave increased antifungal activity.

Some positive points about these studies are; (1) they explored the well-known and widely used traditional plant (*P. betle*) and tested it against a wide range of fungal species with known pathogenicity in human, veterinary and agriculture; (2) *P. betle* is easy to obtain because it is already known and widely used; and (3) the study of potential antifungal property of *P. betle* is encouraged for further usage of these already widely used plant. It means that it will have a great herb-medicine potential and market value that may result in a big impact, for both scientific community and the society as the end user.

The 'back to the nature' issue, failure of synthetic antifungal against clinically resistant species and the fact that it is already well known and widely used as a traditional plant among people in Southeast Asia region, give a strong and positive image to the antifungal properties of *P. betle*.

The negative side of these studies, from the systematic review and meta-analysis point of view, are (1) the studies limited to some fungi only, except the study of Himratul-Aznita *et al.* (2011) and Ali *et al.* (2010) that used numbers of pathogenic fungi; (2) methods were the same so it is very difficult to compare the results due to possible bias; (3) number of species tested are limited; and (4) number of testing are also limited due to the limited number of species tested. These points are great challenges for the next studies in exploring more deeply the antifungal properties of *P. betle*.

Most of these studies are preliminary studies with the great potential for further exploration and analysis. Exploring and characterizing chemical composition of *P. betle* and its pharmacoiological active site, will be a great challenge for future studies.

CONCLUSION

Characterization and identification of effective antifungals from traditional based herb-medicine are very important. The antifungal properties of *Piper betle* Linn is shed a light on this perspective. Further studies are still needed to make it scientifically sound and prove the antifungal properties.

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