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The Spray of Pegagan Leaf Extract as an Antifungal of Vulvovaginal Candidiasis: A Narrative Review

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ABSTRACT

Vulvovaginal Candidiasis (VVC) is a type of infection caused by the fungus Candida spp. The treatment of candidiasis usually uses antifungal drugs against Candida albicans. Pegagan (Centella asiatica (L.) Urban) is one of wild plants that have been used by the community as a drug. The secondary metabolite compounds found in pegagan, such as triterpenoids, alkaloids, flavonoids, and saponins can act as antifungal agents. A literature review of national, international journals and digital books originated from various sites was carried out online. The result of the narrative analysis showed that the ethanol extract of pegagan leaves with a concentration of 75 x 10^3 ppm can inhibit the growth of C. albicans. The results of the toxicity prediction with three parameters showed that the active compounds of pegagan leaves are weak inhibitors, non-carcinogenic and in the toxicity test, it at most belongs to category III. In addition, the spray formulation with a concentration of 1% (w/w) of pegagan leaf extract was found to be safe and non-irritant to skin.

Keywords: *Antifungal, Candida, Pegagan, Vulvovaginal candidiasis*

1. INTRODUCTION

The feminine area is one of the sensitive parts of women which is prone to health problems, such as infections caused by bacteria, fungi, or parasites. Vulvovaginal candidiasis (VVC) is a type of infection caused by fungus Candida spp. VVC is characterized by infection of the genital mucosa, particularly the vulva and vagina. Common clinical symptoms found are itching, burning, pain, and redness which is often accompanied by whitish vaginal discharges (Willems *et al*. 2020).

Vulvovaginal candidiasis is a type of infection in feminine area that often occurs, especially to women of reproductive age. It is estimated around 70-75% of reproductive age women have experienced VVC once in their lifetime and 40-50% of them tend to experience recurrence (Brandolt *et al*. 2017). According to Willems *et al*. (2020), repeated VVC which occurs three times a year is experienced by almost 8% of women globally.

Almost 80-90% of vulvovaginal candidiasis is caused by *Candida albicans* which is a normal microbiota of the vagina. The infection occurs usually caused by various predisposing factors that support the growth of fungi (Willems *et al.* 2020). According to Brandolt *et al*. (2017), several exogenous factors that influence are the increase of climate, heat, and humidity and poor hygiene.

The treatment of candidiasis usually uses antifungal drugs on *Candida albicans.* There are four categories of commonly used antifungal drugs, namely echinocandins, polyenes, azoles, and fluoro-pyrimidines. Excessive use of these drugs can cause side effects, such as nausea, vomiting, diarrhea, and even resistance. Amphotericin B and nystatin in long-term use can also cause kidney damage (Ksiezopolska and Galbadon 2020).

Pegagan (*Centella asiatica* (L.) Urban) is one of wild plants that have been used by the community as a medicinal plant either in the form of fresh, dry, or concoction. The secondary metabolite compounds found in pegagan, includes triterpenoids, alkaloids, flavonoids, and saponins. The saponin compounds in pegagan extract are known to have antifungal activities and hinder the growth of microbes by destroying the cell membrane of the organism's tissue. The terpenoid can interfere with the fungal cell wall by inhibiting the synthesis of 1,3-β-D-glucan for the fungal cell to become lysis (Gintjee *et al*. 2020). Triterpenes are a heterogeneous group of bioactive compounds with a structure consisting of triterpene agiterones (sapogenins) and one or more sugars that bind to acetal glycosidic (ester). Triterpenoid compounds are bioactive which can inhibit the growth of microbes including fungi (Yusuf *et al*. 2017). Based on the content of secondary metabolites, pegagan leaf extract has the potential as an antifungal particularly on *Candida albicans*. Therefore, the spray of pegagan leaf extract is expected to be a practical and effective preparation in overcoming vulvovaginal candidiasis.

Studies on pegagan leaves extract and their bioactive potential have been conducted. However, there are no reviews that specifically analyze the role of focused on analyzing the antifungal potential of pegagan leaf extract against the fungus *Candida albicans* which causes vulvovaginal candidiasis and observing a spray dosage formulation for its application.

2. METHODOLOGY

The methods used are review of literature in the form of national, international journals and digital books originated from various sites, such as ResearchGate, PubMed, Science Direct, NCBI, Elsevier, and Gramedia Digital. Other than that, predictive analysis of active compounds is carried out using the PubChem and admetSAR1 sites to support the data obtained. The keywords used in the literature searching process, namely the

Candida cell membrane, Centella asiatica active compound, inhibition of Candida albicans, toxicity, pegagan spray and skin irritation.

3. RESULT AND DISCUSSION

Pathogenesis of Vulvovaginal Candidiasis

Vulvovaginal candidiasis (VVC) is a superficial infection mostly caused by *Candida albicans*. Broadly speaking, the process of VVC starts from the presence of predisposing factors that make the *C. albicans* easier to attach to mucosal epithelial cells to form colonization. Furthermore, the fungus will release keratolytics which hydrolyze the phospholipids of the epithelial cell membrane, thereby facilitating invasion of the tissue. In the tissue, *C. albicans* will secrete neutrophil chemotactic factors which cause acute inflammatory reactions and manifest as areas of hyperemia or erythema in the vulva and vaginal mucosa. The keratolytic substances that are released by *Candida* will continue to damage the mucosal epithelium, causing shallow ulcers which become heavier by scratching, resulting in erosion. The rest of the necrotic tissue, epithelial cells, and fungi will form white lumps called whitish vaginal discharge (Willems *et al*. 2020; Brandolt *et al*. 2017). Pathogenesis of *C. albicans* is strongly influenced by changes in the commensal form of fungi into hyphae during the colonization process (Mba and Nwaze, 2020).

Antifungal Mechanism of Action

The cell wall of a fungus is composed of mannoproteins, β-glucans matrix, and a phospholipid bilayer whose main component is ergosterol (Freiesleben and Jager 2014). According to Ksiezopolska and Gabaldon (2018), *C. albicans* cell walls and membranes are common targets for commercial antifungal drugs.

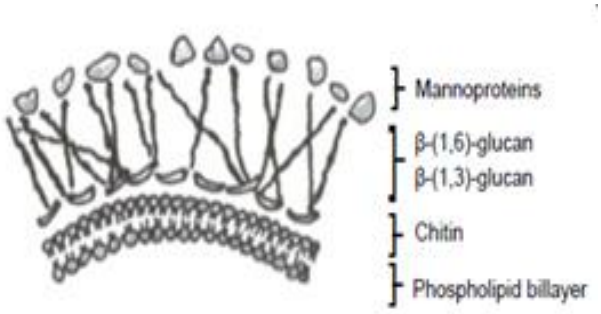

Figure 1 The structure of the cell walls and membranes of fungi (Freiesleben and Jager 2014).

There are four categories of commonly used antifungal drugs, namely echinocandins, polyenes, azoles, and fluoro-pyrimidines (Kaushik dan Kest 2018). Each group has a different procedure. The echinocandins group can influence the biosynthesis of cell walls by inhibiting the action of the enzyme 1,3 – β – glucan synthase (Kaushik dan Kest 2018). Polyenes affect cell membrane integrity by binding to ergosterol (Khan et al 2013). The azoles group inhibits the synthesis of ergosterol (Kaushik dan Kest 2018) and fluoro-pyrimidines which target RNA synthesis and DNA replication (Mahmoud et al 1999).

The Active Compounds of Pegagan Leaf

According to the results of a review by Gray *et al.* (2018), there are 57 active compounds of pegagan leaves which can be seen in (Table 2). A literature study of pegagan leaves extracted with different solvents (Table 3) shows different active compound contents due to the influence of solvent polarity. A compound will dissolve in a solvent that has the same polarity (Leksono *et al*. 2018). Among the six solvents used, ethanol produced the maximum bioactive compound, while hexane produced the minimum bioactive compound. The content of active compounds of pegagan plant is very high and has an important role in medicinal

applications, namely triterpenes (Senthilkumar 2018). Triterpene in pegagan contains many compounds including a siatic acid, madecassic acid, asiaticoside, madecassoside, brahmoside, brahmic acid, brahminoside, thankiniside, isothankunisode, centelloside, madasiatic acid, centic acid, and cenelli acid (Seevaratnam *et al*. 2012). Apart from triterpenes, pegagan also contains high total phenolics derived from flavonoid derivatives, such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferol, castillicetin, and myricetin (Orhan 2012).

Terpenoids can interfere with the fungal cell wall formation by inhibiting the synthesis of 1,3-β-D-glucan for the fungal cells to become lysis (Gintjee *et al*. 2020). Derivative compounds, such as saponins, exhibit antifungal activity by damaging the cell membrane of the fungus (Freiesleben and Jager 2014). Flavonoid is a substance that is known to have antibacterial and antifungal properties (Mickymaray 2019). In general, the way flavonoids work in inhibiting fungal growth includes disrupting the integrity of cell membranes or mitochondrial function and inhibiting cell wall formation, cell division, and RNA and protein synthesis (Al Aboody and Mickymaray 2019).

Flavonoid derivatives, such as quercetin, have been reported as strong inhibitors of the growth of *Candida albicans* (Li *et al*. 2012). Research by Bitencourt *et al*. (2013), also stated that quercetin has anti-fungal properties and works synergistically with fluconazole in inhibiting the action of the fatty acid synthase enzyme which plays an important role in the synthesis of endogenous fatty acids in fungal cell membranes. Apigenin is known to have antifungal activity by inhibiting biofilm formation and stimulating disruption of the fungal cell membrane resulting in a decrease in cell size and leakage of intracellular components (Lee *et al*. 2018). Myricetin and kaempferol inhibit fungal growth through inhibition of nucleic acid synthesis (Cassetta *et al*. 2017).

The Inhibition of *Candida albicans*

The inhibition of *Candida albicans* by pegagan leaf extract (Table 3) generated with various solvents, such as hexane, chloroform, ethyl acetate, ethanol, petroleum ether, and distilled water. A comparison to the inhibition of *Candida albicans* is nystatin, one of the commercial antifungal drugs commonly used to inhibit *Candida albicans*. Most of the extracts of pegagan show an inhibitory power against *Candida albicans*, except at concentrations of 62.5 ppm and 25 x 10^3 ppm from various extracts and hexane extracts with a concentration of 50 x 10^3 diameter of the antifungal inhibition zone are zero.

The comparison for the inhibition of *Candida albicans* called nystatin shows the inhibition zone diameter of 17.35 mm (Balafif *et al*. 2017). Pegagan leaf extract which has an inhibition zone diameter approaching the nystatin inhibition zone, or even higher, are the ethanol extract of pegagan leaves with a concentration of 75 x 10^3 ppm resulted in 17.5 mm inhibition, the ethanol extract of pegagan leaves with a concentration of 100 x 10^3 ppm produced 21.5 mm inhibition, and the ethyl acetate extract of pegagan leaves with a concentration of 100 x 10^3 ppm generated in 18.4 ppm inhibition.

According to Senthilkumar (2018), the ethanol extract of pegagan shows maximum inhibition on *Candida albicans*, causing at a lower concentration of 75 x 10^3 ppm, the ethanol extract of pegagan has an inhibition zone diameter that is scarcely different from the commercial drug, nystatin. Meanwhile, the ethanol and ethyl acetate extract of pegagan leaves have a concentration of 100 x 10^3 ppm. Even though it has a higher inhibition zone diameter, the required concentration is high.

Table 1 Phytochemicals of Pegagan Leaves (Gray *et al*., 2018)

No	Active Compound	Source
1	Arjunolic acid	(Azerad 2016)
2	Asiaticoside D	(Azerad 2016)
3	Asiaticoside E	(Azerad 2016)
4	Asiaticoside F	(Azerad 2016)
5	Asiaticoside G	(Azerad 2016)
6	Centellasaponin A	(Azerad 2016)
7	Centellasaponin B	(Azerad 2016)
8	Centellasaponin C	(Azerad 2016)
9	Centellasaponin D	(Azerad 2016)
10	Centelloside E	(Azerad 2016)
11	Centelloside D	(Azerad 2016)
12	Chebuloside II	(Azerad 2016)
13	Scheffuroside B	(Azerad 2016)
14	Scheffuroside F	(Azerad 2016)
15	Quadranoside IV	(Azerad 2016)
16	Centellasapogenol A	(Azerad 2016)
17	Asiatic acid	(Brinkhaus *et al*. 2000; Jamil *et al*. 2007)
18	Asiaticoside	(Brinkhaus *et al*. 2000)
19	Asiaticoside B	(Brinkhaus e*t al*. 2000)
20	Madecassic acid, brahmic acid	(Brinkhaus *et al*. 2000)
21	Madecassoside	(Brinkhaus *et al*. 2000)
22	Methyleugenol	(Brinkhaus *et al*. 2000)
23	Terminolic acid	(Brinkhaus *et al*. 2000)
24	Chavicol	(Brinkhaus *et al*. 2000)
25	Myrcene	(Brinkhaus *et al*. 2000; Oyedeji and Afolayan 2005)
26	Eugenol acetate	(Brinkhaus *et al*. 2000)
27	Castillicetin	(Chandrika and Prasad Kumarab 2015)
28	Castilliferol	(Chandrika and Prasad Kumarab 2015)
29	Myricetin	(Chandrika and Prasad Kumarab 2015)
30	Patuletin	(Chandrika and Prasad Kumarab 2015)
31	Stigmasterol	(Chandrika and Prasad Kumarab 2015)
32	Kaempferol	(Devkota *et al*. 2010)
33	Campesterol	(Jamil *et al*. 2007)
34	3-Epimaslinic acid	(Jamil *et al*. 2007; Yoshida *et al*. 2005)
35	Asiaticoside C	(James and Dubery 2009)
36	Brahminoside B	(James and Dubery 2009)
37	Neochlorogenic acid (5-O-Daffeoylquinic acid)	(Long *et al*. 2012)
38	Chlorogenic acid (3-O-Caffeoylquinic acid)	(Long *et al*. 2012)
39	Cryptochlorogenic Acid, (4-O-Caffeoylquinic acid)	(Long *et al*. 2012)

Continued Table 1 Phytochemicals of Pegagan Leaves (Gray *et al*., 2018)

No	Active Compound	Source
40	1,3-Dicaffeoylquinic acid	(Long *et al*. 2012)
41	1,5-Dicaffeoylquinic acid	(Long *et al*. 2012)
42	3,4-Dicaffeoylquinic acid	(Long *et al*. 2012)
43	3,5-Dicaffeoylquinic acid	(Long *et al*. 2012)
44	4,5-Dicaffeoylquinic acid	(Long *et al*. 2012)
45	Epicatechin	(Mustafa *et al*. 2010)
46	Catechin	(Mustafa *et al*. 2010)
47	alpha-Humulene	(Oyedeji and Afolayan 2005)
48	Bicyclogermacrene	(Oyedeji and Afolayan 2005)
49	β-Caryophyllene	(Oyedeji and Afolayan 2005)
50	Germacrene B	(Oyedeji and Afolayan 2005)
51	Quercetin	(Sangwan *et al*. 2013)
52	Rutin	(Sangwan *et al*. 2013)
53	Naringin	(Sangwan *et al*. 2013)
54	Pomolic Acid	(Yoshida *et al*. 2005)
55	Sitosterol	(Yoshida *et al*. 2005)
56	Corosolic acid	(Yoshida *et al*. 2005)
57	Ursolic acid	(Yoshida *et al*. 2005)

Toxicity Prediction Analysis

The toxicity analysis of 57 active compounds of pegagan leaves showed that there were 40 active compounds detected by PubChem which were then analyzed by the admetSAR site. The toxicity analysis focuses on three parameters, namely carcinogenicity, Human ether-a-go-go-related gene (hERG), and acute oral toxicity. Carcinogenicity is the ability of a substance or compound to form cancer (Astutiningsih *et al*. 2010). The results of the toxicity test analysis (Table 4) showed that 40 active compounds of pegagan leaves are non-carcinogenic indicating that they do not have the potential to form cancer.

Human ether-a-go-go-related gene (hERG) is a gene related to encoding the pore formation subunit of the K^+ channel which plays an important role in the repolarization of the heart muscle. The inhibition or reduction of hERG activities causes loss consciousness and sudden death that occurs in patients with cardiac ischemia (Lamothe *et al.* 2016). The results of the toxicity prediction in (Table 4) showed that the active compounds of pegagan leaves are weak inhibitors of hERG.

Acute oral toxicity is an essential test to observe the toxicity of a drug or compound when it enters the digestive system within a certain time after giving a single dose (Zulfiana, 2014). The level of toxicity is divided into 5 categories based on the LD_{50} score, including category 1 (≤5 mg/kg), category 2 (5 mg/kg < LD50 ≤ 50 mg/kg), category 3 (50 mg/kg < LD50 ≤ 300 mg/kg), category 4 (300 mg/kg < LD50 ≤ 2000 mg/kg) and category 5 (2000 mg/kg < LD50 ≤ 5000 mg/kg) (Son and Yen, 2014). The results of the toxicity prediction show that all ligands belong to category III, except for campesterol, sitosterol, and stigmasterol which are classified as category I, quercetin, myricetin, kaempferol, and chavicol are classified as category II and Epicatechin belongs to category IV.

Table 2 The Active compounds of Pegagan Leaves and inhibition of *Candida albicans*

Sample	Active Compound	Concentration (ppm)	Antifungal Inhibition Zone Diameter (mm)	Source
Pegagan Leaf Hexane Extract	Alkaloid, steroid, and flavonoid	25×10^3	0	(Dash *et al.* 2020); (Senthilkumar 2018)
		50×10^3	0	
		75×10^3	10.6	
		100×10^3	13.2	
		500×10^3	11	
Pegagan Leaf Chloroform Extract	Terpenoid, quinone, alkaloid, carbohydrate, steroid, flavonoid, and phenol	25×10^3	10.7	(Dash *et al*. 2020); (Senthilkumar, 2018); (Yadav *et al*. 2017)
		50×10^3	12.4	
		75×10^3	14.2	
		100×10^3	15	
		500×10^3	15	
Pegagan Leaf Ethyl Acetate Extract	Alkaloid, saponin, quinone, flavonoid, tannin, phenol, and terpenoid	25×10^3	0	(Senthilkumar 2018); (Yadav *et al*. 2017)
		50×10^3	10.7	
		75×10^3	13.4	
		100×10^3	18.4	
Pegagan Leaf Ethanol Extract	Alkaloid, glycoside, flavonoid, phenol, saponin, tanin, terpenoid, and steroid	62.5	0	(Dash *et al*. 2020); (Hapsari *et al*. 2017); (Jangtap *et al*. 2009); (Senthilkumar 2018); (Yadav *et al*. 2017)
		125	9	
		250	12	
		500	12	
		1×10^3	16	
		25×10^3	11.4	
		50×10^3	14.6	
		75×10^3	17.5	
		100×10^3	21.5	
		500×10^3	15	
Pegagan Leaf Petroleum Ether Extract	Alkaloid, flavonoid, terpenoid, and quinone	62.5	0	(Dash *et al*. 2020); (Jangtap *et al*. 2009); (Jayaprakash and Nagarajan 2016); (Yadav *et al*. 2017)
		125	9	
		250	11	
		500	12	
		1×10^3	15	
		500×10^3	13	
Pegagan Leaf Aquades Extract	Flavonoid, triterpenoid, saponin, alkaloid, and tannin	62.5	0	(Dash *et al*. 2020); (Ismaini 2011); (Jangtap *et al*. 2009); (Wiendarlina *et al*. 2018)
		125	9	
		250	10	
		500	12	
		1×10^3	12	
		500×10^3	10	
Nystatin (Comparison)	-	50	17.35	(Balafif *et al*. 2017)

Table 3 Toxicity prediction results

Active	Inhibisi Human Ether-A-Go-Go Related Gene (herG)		Carcinogenicity		Acute Oral Toxicity	
	Category	Score	Category	Score	Category	Score
alpha-Humulene	Weak inhibitor	0.9169	Non-Carcinogenic	0.6532	III	0.6889
Arjunolic acid	Weak inhibitor	0.9707	Non-Carcinogenic	0.9574	III	0.8032
Asiatic acid	Weak inhibitor	0.9579	Non-Carcinogenic	0.9599	III	0.7783
Asiaticoside	Weak inhibitor	0.9359	Non-Carcinogenic	0.9618	III	0.6957
Asiaticoside F	Weak inhibitor	0.9359	Non-Carcinogenic	0.9618	III	0.6947
Bicyclogermacrene	Weak inhibitor	0.9326	Non-Carcinogenic	0.7061	III	0.7166
β-Caryophyllene	Weak inhibitor	0.9225	Non-Carcinogenic	0.6863	III	0.82
Campesterol	Weak inhibitor	1.773	Non-Carcinogenic	0.932	I	0.5508
Catechin	Weak inhibitor	0.9666	Non-Carcinogenic	0.9539	IV	0.6433
Centellasapogenol A	Weak inhibitor	0.9707	Non-Carcinogenic	0.9574	III	0.8032
Chavicol	Weak inhibitor	0.717	Non-Carcinogenic	0.7331	II	0.5373
Chlorogenic acid	Weak inhibitor	0.9862	Non-Carcinogenic	0.9341	III	0.7775
Corosolic acid	Weak inhibitor	0.9796	Non-Carcinogenic	0.9552	III	0.647
Cryptochlorogenic Acid	Weak inhibitor	0.9862	Non-Carcinogenic	0.9341	III	0.7775
1,3-Dicaffeoylquinic acid	Weak inhibitor	0.9858	Non-Carcinogenic	0.9247	III	0.7458
1,5-Dicaffeoylquinic acid	Weak inhibitor	0.9858	Non-Carcinogenic	0.9247	III	0.7458
3,4-Dicaffeoylquinic acid	Weak inhibitor	0.9862	Non-Carcinogenic	0.9341	III	0.7775
3,5-Dicaffeoylquinic acid	Weak inhibitor	0.9847	Non-Carcinogenic	0.9213	III	0.7686
4,5-Dicaffeoylquinic acid	Weak inhibitor	0.9862	Non-Carcinogenic	0.9341	III	0.7775
Epicatechin	Weak inhibitor	0.9666	Non-Carcinogenic	0.9539	IV	0.6433

Continued Table 3 Toxicity prediction results

Active	Inhibisi Human Ether-A-Go-Go Related Gene (herG)		Carcinogenicity		Acute Oral Toxicity	
	Category	Score	Category	Score	Category	Score
3-Epimaslinic acid	Weak inhibitor	0.9796	Non-Carcinogenic	0.9552	III	0.647
Eugenol acetate	Weak inhibitor	0.9535	Non-Carcinogenic	0.8445	III	0.8552
Germacrene B	Weak inhibitor	0.8372	Non-Carcinogenic	0.6421	III	0.7428
Kaempferol	Weak inhibitor	0.9795	Non-Carcinogenic	0.9363	II	0.6238
Madecassic acid, brahmic acid	Weak inhibitor	0.9579	Non-Carcinogenic	0.9599	III	0.7783
Madecassoside	Weak inhibitor	0.9359	Non-Carcinogenic	0.9618	III	0.6947
Methyleugenol	Weak inhibitor	0.8488	Non-Carcinogenic	0.8119	III	0.9019
Myrcene	Weak inhibitor	0.865	Non-Carcinogenic	0.5684	III	0.803
Myricetin	Weak inhibitor	0.9781	Non-Carcinogenic	0.945	II	0.7348
Naringin	Weak inhibitor	0.9786	Non-Carcinogenic	0.9539	III	0.5734
Neochlorogenic acid (5-O-Daffeoylquinic acid)	Weak inhibitor	0.9862	Non-Carcinogenic	0.9341	III	0.7775
Patuletin	Weak inhibitor	0.9756	Non-Carcinogenic	0.9505	III	0.6309
Pomolic Acid	Weak inhibitor	0.9601	Non-Carcinogenic	0.9473	III	0.8579
Quadranoside IV	Weak inhibitor	0.929	Non-Carcinogenic	0.9617	III	0.7112
Quercetin	Weak inhibitor	0.9781	Non-Carcinogenic	0.945	II	0.7348
Rutin	Weak inhibitor	0.9814	Non-Carcinogenic	0.9608	III	0.5971
Sitosterol	Weak inhibitor	0.8027	Non-Carcinogenic	0.9182	I	0.4287
Stigmasterol	Weak inhibitor	0.8027	Non-Carcinogenic	0.9182	I	0.4287
Terminolic acid	Weak inhibitor	0.9707	Non-Carcinogenic	0.9574	III	0.8032
Ursolic acid	Weak inhibitor	0.9582	Non-Carcinogenic	0.9394	III	0.8316

The Spray Formulation of Pegagan Leaf Extract

The spray formulation used refers to the research of Sawatdee *et al.* (2016) and the results of a literature review on the inhibition of *Candida albicans*. The formulation uses pegagan leaves ethanol extract with a concentration of 75 x 10^3 ppm. The additional ingredients are HP-β-CD, eudragit E100 and copovidone as a polymer coating, glycerol and PEG 400 as a humectant, and ethanol and distilled water as solvents.

The process of making spray begins by mixing 1% (w/w) the extract and 2% HP-β-CD into 7% distilled water. Furthermore, 2% eudragit E 100 and 6% copovidone are dissolved in 70% absolute ethanol. Both solutions are mixed and stirred at a speed of 300 rpm until it is clear. After that, add 10% PEG 400 and 5% glycerol and stir until the mixture is homogeneous. This formulation is used as a reference as it has the best physical properties (appearance, pH value and viscosity) and spreadability (Sawatdee *et al.*, 2016).

Skin Irritation Analysis

The analysis of the impact of spray from pegagan leaf extract on the skin carried out by Sawatdee *et al*. (2016) showed that no edema was detected in primary skin irritation studies on selected formulations which also have the potential to be used as formulations for spraying pegagan extract for *Candida albicans* antifungal. Therefore, pegagan leaf extract was found to be safe and non-irritant to skin.

In conclusion, The ethanol extract of pegagan leaves with a concentration of 75 x 10^3 ppm had the best inhibition against *Candida albicans* at 17.55 mm. The content of secondary metabolites of ethanol extract of pegagan, such as flavonoids, terpenoids, alkaloids, and saponins, have antifungal properties that work in different ways. The spray of pegagan leaf extract with an extract concentration of 1% (w/w) and several additional ingredients, such as HP-β-CD, eudragit E100, copovidone, glycerol, PEG 400, ethanol, and distilled water have the physical properties (appearance, pH value and viscosity) and the best dispersibility and are safe for the skin.

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