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The Study on The Effect of Phytochemical Properties in Aloe vera Extract To Oral Candida in Cancer Survivor

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Abstract

Ethanol extract of aloe vera has known as antifungal for oral Candida Spp., because of its phytochemical properties. The purpose of this study is to investigate the effect of phytochemical properties in Aloe vera extract to oral Candida in cancer survivors who was under chemotherapy. The methods in this study are Aloe vera were extracted using maceration technique and phytochemical screening has done. Aloe vera extract has given as single use oral rinse. Candida spp. was collected before and after rinsing with aloe vera extract, using Oral Rinse Concentrate technique and inoculated at Sabouraud's Dextrose Agar (SDA). The number of candida's colony forming units were counted. The result of this research showed that Aloe vera extract has phytochemical properties such as alkaloid, flavonoid, steroid, saponin, polyphenol, quinone and triterpenoid and reduce the number of oral Candida's colony were 37.45 % and 26.47% in average. The conclusion of this study is the Aloe vera extract can be used as antifungal agents.

Keywords: Phytochemical Properties, Aloe vera Extract, Oral Candida, Antifungal, Cancer Survivor

Nomenclature

ATCC	American Type Culture Cells
AVL	Aloe vera leaf , the extract only made from the green skin of Aloe vera plants/leaf
AVG	Aloe vera gel, the extract only made from inner gel of Aloe vera plants/leaf
AVW plants/leaf	Aloe vera whole, the extract made from the green skin and the inner gel of Aloe vera
CFU/ml	Colony Forming Units/ml saliva.
PBS	Phosphate Buffer Saline.
v/v	Ratio concentration (volume/volume).

1. Introduction

Cancer patients can suffer oral toxic effects secondary to antineoplastic therapy in the form of chemotherapy, conditioned by a range of factors, including the high cell turnover rate of the oral mucosa, the diversity and complexity of the oral micro flora, and soft tissue trauma during normal oral function. Chemotherapy as one of the cancer treatment also induce the increasing number of oral candida's colonization.[1] Oral candidiasis is one of the oral complication in the treatment of cancer patients and being preceded with the colonization of Candida species in the mouth. The symptoms of oral candidiasis have negative impact on the quality of life and can impair nutritional intake of the cancer survivor.[2]

Lalla, et al., has reported that the prevalence of oral candidiasis was 7.5% pre-treatment, 39.1% during treatment, and 32.6% after the end of cancer therapy. The prevalence of oral candidiasis was 38% and the prevalence of oral colonization with fungal organisms was 72.8%, during chemotherapy.[1]

Treatment for oral candidiasis is antifungal agents, such as Polyene antibiotics and azoles, but recently the incidences of resistance has increased. An epidemiology study conducted by Sobelson S., et al., revealed that the overall resistance to azoles was 28.2% from 266 respondents. Resistance to specific drugs was also seen for fluconazole (4.5%), itraconazole (11.7%), ketoconazole (11.3%), voriconazole (0.75%) and caspofungin (41.1%), but none in amphotericin B or nystatin.[3] Thus, the development of an alternative or a new antifungal agent is important.

Aloe vera has known as antifungal because of its phytochemical properties. A comparative study of the antifungal effect of Aloe vera that has been published by Agarry O.O., et al., revealed that zone of inhibitory to Candida albicans of Aloe vera leaf extract (AVL) was 3.00 mm and 0.00 mm for Aloe vera gel (AVG).[4] Another study on AVL extract has been found the minimal fungicidal concentration for Candida albicans ATCC 10231 strains was 75% and it is important for clinical treatment.[5] There is a few study has been done for the effect of Aloe vera whole extract (AVW) to Candida species.

The anthraquinones component in Aloe vera such as aloin and aloe emodin has been suggested as an antifungal. A high content of both 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and their

glycosides (aloins) has been demonstrated in Aloe vera, and presents antibacterial activity by inhibition of nucleic acid synthesis in microbes, inhibit cell growth and germ tube formation by Candida albicans.[6]

The aim of this study was to investigate the effect of phytochemical properties in the Aloe vera extract and compare both the effect of the Aloe vera leaf (AVL) and Aloe vera whole (AVW), as a single use oral rinse in reducing the Colony Forming Units of Candida species. Dental and medical condition of the patients being analyzed due to the effectiveness of those extract in reducing the Colony Forming Units of Candida species.

2. Materials and Methods

2.1 Collection of Plant materials:

Aloe vera plants picked up from an organic garden near Padjadjaran University, Jatinangor - Sumedang, Indonesia. This plants has been identified as Aloe barbadensis Miller or Aloe vera. The selected Aloe vera were 50 – 60 cm in length, 10 – 12 cm in width and the weight was 420 grams in average. There is two parts in Aloe vera leaves, the outer skin which color is green and the inner gel which color is clear, as described by Figure 1. The outer skin of Aloe vera leaves then being extracted as Aloe vera leaf extract (AVL) and the whole Aloe vera including the outer and inner part being extracted as Aloe vera whole extract (AVW).

2.2 Preparation of Aloe vera Extract

Both of the fresh Aloe vera, AVL and AVW then macerated in 96% ethanol. One until two days after, the macerated Aloe vera was filtered through Wattman no 1 filter paper, and then evaporated using Rotary Evaporator (Rotavapor R-215, Buchi) at 30-40 degree Celsius. The extract was preserved aseptically in a brown bottle at 5 degree Celsius until used as described by the refferences.[5,7,8] Screening of phytochemical components also done for both of the extract. In this study we used Aloe vera extract dissolved with aquadest until 75% (v/v) in concentration reached.

Fig. 1. (a) Aloe vera outer skin (AVL); (b) Aloe vera whole (AVW).

2.3 Ethical clearance, Saliva Collection and Preparation:

The procedures in this study was approved by the Medical Ethics Committee from Hasan Sadikin Hospital, Bandung, Indonesia. Inclusion criteria for the subject of this study were head and neck cancer patients who was undergoing 5-FU and Cisplatin/Carboplatin chemotherapy. All of the patients were required to sign an informed consent form prior to entry into the study. Four patients rinsed with 10 ml AVL and 4 patients rinsed with 10 ml AVW, the rinsing time was between 1-3 minutes.

The concentrated oral rinse methods was used to isolate Candida species from 8 patients that fulfilled the criteria, as described by the refferences.[9,10] The patients have to swish with 10 ml of Phosphate Buffer Saline (PBS, pH = 7,2) for 1 minute, and then the saliva collected in a sterile medium transport. Saliva from the patients collected twice, before and after rinsing with the extract.

Candida species were cultured on Sabouraud's dextrose agar plates and being incubated for 24-48 hours in 37 degree Celsius. The total amount of Candida's colony forming units being counted as CFU/ml concentrated saliva.

3. Results and Discussion

The screening of phytochemical properties of AVL and AVW extracts were done. The results were shown in Table 1. The phytochemical properties of AVL were alkaloids, flavonoids, steroids, saponins, polyphenols, triterpenoids and quinones, but tanins were absent. In the other side, the phytochemical properties of AVW were similar to AVL with none of alkaloids and tanins.

The 75% (v/v) of AVL extract and 75% (v/v) AVW extract being tested as an oral rinse single use to the patients. The colonization of Candida species in this study before rinsing with AVL extract was 31.875

CFU/ml in average, then after rinsing decreased until 21.775 CFU/ml in average. The reduction of oral candida species colonization after rinsing with 75% AVL extract was 37,45%, as described in Table 2.

Table 1. Qualitatively Analysis of Phytochemical properties of Aloe vera Extract

Phytochemical properties	Aloe vera Leaf (AVL) extract	Aloe vera Whole (AVW) extract
Alkaloids	+	-
Flavonoids	+	+
Steroids	+	+
Tanins	-	-
Saponins	+	+
Polyphenols	+	+
Triterpenoids	+	+
Quinones	+	+

In the other side, the colonization of Candida species before rinsing with AVW extract was 26.975 CFU/ml in average, then after rinsing decreased until 19.525 CFU/ml in average. The reduction of oral candida species colonization after rinsing with 75% AVW extract was 26,47%, as described in Table 3.

Table 2. Colony Forming Units (CFU/ml) of Candida Spp.
Before and After Rinsing with 75% Aloe vera Leaf Extract (AVL)

Respondents (n = 8)	Extract, rinsing time	CFU/ml Before Rinsing (x100)	CFU/ml After Rinsing (x100)	Reduction of CFU/ml	
				CFU/ml (x100)	(%)
1	AVL, 1 minute	151	47	104	68.87
2	AVL, 2 minutes	201	127	74	36.82
3	AVL, 2 minutes	215	177	38	17.40
4	AVL, 3 minutes	708	520	188	26.55
	Mean	318.75	217.75	101	37.45

Table 3. Colony Forming Units (CFU/ml) of Candida Spp.
Before and After Rinsing with 75% Aloe vera Whole Extract (AVW)

Respondents (n = 8)	Extract, rinsing time	CFU/ml Before Rinsing (x100)	CFU/ml After Rinsing (x100)	Reduction of CFU/ml	
				CFU/ml (x100)	(%)
5	AVW, 1 minute	192	128	64	33.33
6	AVW, 2 minutes	316	142	174	55.06
7	AVW, 2 minutes	179	153	26	14.52
8	AVW, 3 minutes	422	358	64	15.16
	Mean	269.75	195.25	74.5	26.47

Saliva sample examination from the patients who had received 75% AVL extract as single oral rinse showed higher reduction in CFU/ml Candida species than 75% AVW extract recipients. These condition are probably due to the alkaloids as one of the phytochemical properties in AVL extract that could not find in AVW extract. The efficacy of the alkaloids as an antimicrobial is significantly high even at low concentration, which indicates a possibility of its use as antifungal.[11]

Study on phytochemical properties of Aloe vera before, showed that anthraquinones has a potential role as antifungal.[6] Another study revealed that the high molecular weight components (MW > 100 kDa) is the most effective fraction of Aloe vera that can increase in macrophage activity against Candida albicans. The acemannan (beta 1,4-acetylated mannan) can be isolated from Aloe vera with 274 – 375 molecular weight.[11] Whilst the mechanism of anti-bacterial activity is still need more investigations, it has been suggested that aloe emodin and aloins induced bacterial cell membranes disruption and the anthraquinone had strong anti-bacterial activity.[12]

It has known that the phenol component in Aloe vera have anti-bacterial agents by disrupting bacterial cell membranes, as well as by denaturing bacterial proteins. Cinnamic acid in Aloe vera is known to inhibit bacterial glucose uptake and ATP production, therefore, inhibiting bacterial growth and the coumaric acid has been shown to inhibit bacterial enzymatic activity. Aloe vera components may also function by selectively modulating the cells of the immune system. Furthermore, acemannan also inhibits bacteria adhering to epithelial cells and establishing an infection.[12]

It is likely that the anti-bacterial activity of Aloe vera extracts in vivo is due to the synergistic effects of multiple bioactive components, functioning through several mechanisms. Then more detailed purification, identification and mechanistic studies of Aloe vera as an antifungal agents are required.[12]

Medical and oral condition of these patients has been analyzed due to the effectiveness of those extract in reducing the Colony Forming Units of Candida species. Almost all of the patients (3 from 4) who had

received AVW extract was during intravenous chemotherapy and they showed less reduction in CFU/ml than the patients who had received AVL extract which was in the between the cycle of chemotherapy. The antineoplastic agents were on the peak concentration in the body while the patients during intravenous chemotherapy, and decreased oral cellular defense mechanism too. This study revealed that the Aloe vera extract as antifungal less effective in this situation (Figure 2).

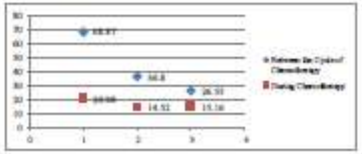

Fig. 2. (blue dots) Patients Between the Cycle of Chemotherapy and received AVL, (red dots) Patients During Chemotherapy and received AVW.

4. Conclusion

We investigated the effect of the phytochemical properties of Aloe vera extract (AVL and AVW) to oral candida species in cancer survivor in order to find a new alternative antifungal agent. It is found that 75% Aloe vera leaf extract (AVL) as a single use oral rinse could reduce more CFU/ml oral Candida species than 75% Aloe vera whole extract (AVW). We also found that the Aloe vera extract become less effective probably due to the receiving intravenous chemotherapy.

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