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Analysis Of Liquid Chromatography- Mass Spectrometer (Lc-Ms) Of Kepyar Leaf Extract 50%As A Denture Disinfection Material In The Inhibition Zone Of Candida Albicans And Surface Roughness Heat Polymerized Acrylic Resin

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Abstract
Background *: The continuous use of dentures that are not well cared for can cause halitosis and inflammation of the oral mucosa. Therefore, maintaining the cleanliness of dentures is very important to prevent contamination of dentures by dirt and microorganisms. A solution to maximize the cleanliness of dentures is to soak them in a disinfectant solution..*
Methods *: The method used was the extraction of dried leaf powder of ricinus communis leaf using ethanol as a solvent by the maceration process and then analyzed using) and LCMS to obtain information on the contents in ricinus communis leaf. The thick castor leaf extract was tested by LC-MS to identify the effective phytochemical compounds as antifungals. Disinfection of denture base samples in chlorhexidine and 50%, castor oil extract to observe Candida albicans inhibition zones*
Results*: The results of the LC-MS test identified the presence of phytochemical compounds belonging to the flavonoid, phenol and terpenoid groups. The average inhibition zones formed in the chlorhexidine treatment group, 50% castor oil extract*
Conclusion*: There is an effect of castor oil leaf extract as a denture disinfection agent on the inhibition zone of Candida albicans.*
Keyword: *Analysis of Chromatograhpy Compounds, Denture Disinfection, Castor Leaf Extract, Inhibition Zone of Candida Albicans, Surface Roughness*

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I. Introduction

A denture is a device that can replace the function of a missing tooth and its surrounding tissues.[1] The components of a denture consist of elements of an artificial tooth and a denture base. The denture base material that is often used today is hot polymerized acrylic resin, because it has good aesthetic advantages, texture and color similar to gingiva, small dimensional changes, relatively cheap price, more comfortable and lighter to use. Hot polymerized acrylic resin has biological, mechanical, chemical and physical properties. The physical properties of hot polymerized acrylic resin are surface hardness, thermal conductivity, dimensional stability and color stability. The mechanical properties of acrylic resin classify it as a more flexible material compared to metals. Chemical properties of hot polymerized acrylic resin slowly absorb water and an equilibrium value of about 2% absorption is reached after a few days or a few weeks depending on the thickness of the denture. Biological properties give the ability of certain microorganisms to colonize the surface of the acrylic denture base. The type of microorganism that is often found on denture bases is Candida albicans. Regular denture disinfection can usually prevent the growth of these unwanted microorganisms and associated clinical problems, such as denture stomatitis.[2]

Instructions after insertion of dentures that must be paid attention to by patients are adequate denture disinfection, so that denture cleanliness can be maintained. Disinfection of dentures that are commonly carried out are mechanical, chemical and combined. Some materials that are categorized as chemical denture disinfection available are non-traditional and traditional materials. Non-traditional ingredients consist of sodium hypochlorite, alkaline peroxide, chlorhexidine. One example of traditional ingredients is betel leaf extract, jatropha leaves and other herbal ingredients.[3]

Research on denture disinfection is still being carried out because until now the ideal denture disinfection

has not been found. The World Health Organization (WHO) recommends substances and materials derived from minerals, animals and plants.[4] At present, disinfection materials have been developed which contain natural ingredients that are easy to obtain with superior biocompatibility and low development costs.

Currently, the material being studied in all fields of health is the jatropha castor (Ricinus communis). Jatropha castor leaf extract (Ricinus communis) has shown antibacterial activity against various pathogenic bacteria. Jatropha castor leaves contain phytochemical compounds such as tannins, terpenoids, saponins, phenols and flavonoids which can work as anti-microorganisms.[5] Research conducted by Sulastri, et al (2015), tested the antimicrobial activity of the methanol extract of jatropha leaves (Ricinus communis l.) against Aspergillus niger using Liquid Chromatography-Mass Spectrophotometer (LC-MS). The results of LC-MS analysis showed the presence of a ricinin compound, which was suspected to be toxic to A. niger, a type of fungus that is the same as Candida albicans. Candida albicans can inhibit its growth due to the mechanism of action of flavonoids by causing disruption of the ethanol permeability of fungal cells. Tannins also have an antifungal mechanism, namely their ability to inhibit the synthesis of chitin which is used for the formation of cell walls in fungi and damage cell ethanol so that fungal growth becomes inhibited.[6]

II. Method

Tools and materials

The tools used are metal master model measuring 10 × 10 × 2 mm, mask gloves, ordinary scales, rubber bowl, spatula, cuvette, lekron, vibrator, acrylic pot, dropping pipette, cement spatula, hydraulic press, rotary grinder, paper sand number 240, 400, 600, 800, 100 gram cotton, filter paper, digital scales, blender, measuring cup, Erlenmeyer flask, autoclave, petri dish, syringe, vortex, Beaker glass, flannel cloth, Incubator oven, 10µL size calibration loop , Bunsen lamp, spirit, hot plate, paper disk, test tube, rotary evaporator, 10 ml vial, marker pen, materials used hot polymerized acrylic resin, castor oil leaves, could mold seal, hard plaster and water, vaseline, plastic cellophane , distilled water, sand paper, cotton buds, candy paper, 70% ethanol, dimethyl Sulfoxide, potato dextrose agar, Candida albicans suspension, sterile 0.9% NaCl solution.

Sample making

The study sample was prepared from hot polymerized acrylic resin, obtained from a 10 × 10 × 2 mm metal master model. Furthermore, making the mold, starting with making hard plaster dough, the ratio of gypsum to water is 100 grams: 30 ml of water for the top and bottom cuvettes. the dough was stirred with a spatula for 20 seconds until it was mixed homogeneously for 30 seconds and then the dough was put into the cuvette that had been prepared above the vibrator. The main model is placed on the gypsum dough which is starting to harden in the cuvette. After it hardens a bit, the gypsum is tidied up and left to stand until it hardens completely. The surface of the cast and cuvette is smeared with Vaseline then the top cuvette is attached and filled with hard plaster dough over the vibrator. After the gypsum has hardened, the cuvette is opened, the main model is removed, the cuvette is poured with hot water to remove the remaining Vaseline until it is clean, after it is dry, spread with cold mold seal, wait for 20 minutes (according to the manufacturer's instructions).

Fill the acrylic resin in the mold. Polymer and monomer are stirred in a porcelain pot with a ratio of 2 gr: 1 ml according to the manufacturer's instructions and wait until the dough reaches the dough stage. The mold that has been smeared with the separator is completely filled with acrylic resin mixture. Plastic cellophane is placed between the top and bottom cuvettes, then closed and pressed gently with a hydraulic press with a pressure of 1000 psi. The cuvette was opened and the excess acrylic was cut off, then the cuvette was closed again, pressing was done with a pressure of 2200 psi. The water bath is filled with water, the temperature and time are set at 70°C for 90 minutes and 100°C for 30 minutes. The cuvettes were removed from the water bath and allowed to cool to room temperature. The final sample was removed from the cuvette, then trimmed to remove sharp edges using a Fraser bur. The samples were then ground with waterproof sand paper with numbers 400, 600 and 1000 under running water using a rotary grinder until they obtained the desired size. Samples were soaked in distilled water for 48 hours before being given treatment to remove residual monomers.

Extract manufacture

Extract preparation, Jatropha leaves are washed with running water. Dry in an open room that is not exposed to direct sunlight to dry. Then grind it into powder using a blender. Jatropha leaf powder was macerated using 70% ethanol with a ratio of 1:10 at room temperature for 5 days accompanied by stirring. The liquid extract was then filtered using filter paper (maserat I). Repeat the extraction process on the dregs using 70% ethanol to obtain macerate II**.**

Preparation of test solutions

Preparation of test solutions with various concentrations using standard mother liquor (LIB). LIB 6 gr/10 ml (Concentration 60%). You need 30 ml LIB, 6x3 = 18 gr. Weigh 18 g condensed castor oil extract and then

dilute it with 30 ml DMSO to produce 30 ml of 60% jatropha leaf extract. The concentration is lowered, 50 ÷ 60 x 10 = 8.3 ml, take 8.3 ml of jatropha leaf extract, then add 1.7 ml of DMSO. Produce 10 ml of jatropha leaf extract with a concentration of 50%?

LC-MS Test Analysis of Jatropha Leaves Extract

Jatropha leaf extract (Ricinus communis) was put into vials and then analyzed by gas chromatography-mass spectrometry, the extract was injected into LC-MS using a capillary column, the initial oven temperature was 200 ℃ and held for 2 minutes, then the temperature was increased 10 ℃/minute until the temperature 285 ℃ hold for 20 minutes. The injection and detector temperature is set at 280℃.

Measurement of Inhibition Zone Against Candida Albicans

Samples were sterilized using an autoclave at 121 ℃ for 1 hour then the samples were rinsed with distilled water, the samples were divided into 2 groups, namely group 1 disinfection with 0.2% Chlorhexidine, group 2 disinfection with Jatropha leaf extract (Ricinus communis), samples were soaked in artificial saliva for 1 hour at 37℃. Then rinsed 2 times using Phosphate Buffered Saline, the sample was contaminated with Candida albicans by placing it in an Erlenmeyer tube containing Candida albicans suspension. Preparation of Candida albicans suspension was carried out by taking 1-2 oses of pure cultured Candida albicans which had been cultured and then mixed with 0.9% NaCl solution until turbidity was obtained according to Mac Farland standards or equivalent to 1x108 CFU/ml. Then incubated for 24 hours at 37ºC, after 24 hours, the sample was removed from the test tube. Each one sample was put into a reaction vial containing 2 ml each, namely Jatropha castor leaf extract (Ricinus communis). Samples were soaked and stored in an incubator at 37℃ for 4 days, then samples were removed from the test tube and rinsed with Phosphatase Buffered Saline (PBS) twice, the sample was put into a test tube containing 10 ml of 0.9% NaCl solution and then vibrated using a vortex vibrating device for 30 seconds to release Candida albicans attached to the sample, paper disks were put into test tubes for the treatment group and control group, Candida albicans isolates in the test tube were taken with a sterile stick and then suspended in 9 mL of 0.9% NaCl , as much as 1 ml of Candida albicans suspension was put into an Erlenmeyer containing PDA liquid, homogenized then poured into a petri dish. Wait until it hardens, each paper disk is removed from the vial and then arranged on top of the hardened PDA media. Incubated for 48 hours at 37 ℃, the diameter of the inhibition zone was measured with a caliper in millimeters.

Statistics

Data analysis used in this study was univariate test analysis to determine the average standard deviation of each group. Analysis of one way Anova test to determine the effect of disinfection of 0.2% chlorhexidine and 50%, castor oil (*Ricinus communis*) leaf extract on the inhibition zone of *Candida albicans* on heat polymerized acrylic resin denture base.

III. Research Result

The diameter of the *Candida albicans* inhibition zone formed on agar media was measured using a calliper in millimeters. The results showed that the diameter of the *Candida albicans* inhibition zone in group A with chlorhexidine as a disinfecting agent, the smallest value was 11.8 mm and the largest value was 14.8 mm. The diameter of the Candida albicans inhibition zone in group B with Jatropha leaf extract concentration of 50% as a disinfecting agent with the smallest value was 13 mm and the largest value was 16.5 mm. The diameter of the Candida albicans inhibition zone in group C with Jatropha leaf extract concentration of 60% as a disinfecting agent with the smallest value was 14 mm and the largest value was 19.7 mm.

The mean value of the Candida albicans inhibition zone was analyzed using the univariant test. The mean value of the Candida albicans inhibition zone in group A was 13.09 ± 1.02 mm. The mean value of the Candida albicans inhibition zone in group B was 14.47 ± 1.249 mm. The mean value of the Candida albicans inhibition zone in group C was 16.755 ± 1.974 mm.

Table 1. Diameter of Inhibition Zone of Candida albicans on Heat Polymerized Acrylic Resin Denture Base After Chlorhexidine Disinfection and Castor Leaf Extract (Ricinus communis)

No.	Diameter Zona Hambat *Candida albicans* (mm)	
	Kelompok A	Kelompok B
1	11,8	13,85
2	13,8	15,2
3	11,95	15,65
4	12,1	14,45
5	12,55	13
6	12,8	15,65
7	13,7	14,3
8	14,8	16,5

9	14,2	13,1
10	13,2	13
$\bar{X}$ ± SD	13,09 ± 1,02	14,47 ± 1,249

The results of the one way Anova test obtained a significance of p = 0.0001 (p <0.05). This shows that there is a disinfection effect of chlorhexidine and Jatropha castor leaf extract (Ricinus communis) concentrations of 50% on the inhibition zone of Candida albicans on hot polymerized acrylic resin denture base.

Table 2. The effect of chlorhexidine disinfection (A) and 50% concentration of castor oil (Ricinus communis) leaf extract (B) on the inhibition zone of Candida albicans on hot polymerized acrylic resin denture bases

Kelompok	Zona Hambat *Candida albicans* (mm)		
	n	*x* ± SD	p
A	10	13,09 ± 1,02	0,0001*
B	10	14,47 ± 1,24	

Table 3. Surface Roughness Value of Hot Polymerized Acrylic Resin Denture Base After Disinfection with Chlorhexidine and 50% Concentration of Ricinus Communis Leaf Extract

No.	Nilai Kekasaran Permukaan Basis Gigi Tiruan Resin Akrilik Polimerisasi Panas (µm)	
	Kelompok A	Kelompok B
1	0,233	0,126
2	0,308	0,147
3	0,151	0,166
4	0,197	0,188
5	0,147	0,148
6	0,183	0,132
7	0,124	0,171
8	0,208	0,166
9	0,21	0,16
10	0,208	0,141
$\bar{X}$ ± SD	0,196 ± 0,051	0,154 ± 0,019

Table 4. The effect of chlorhexidine disinfection (A) and 50% concentration of castor oil (Ricinus communis) leaf extract (B) on the surface roughness of hot polymerized acrylic resin denture bases

Kelompok	Kekasaran Permukaan		
	n	*x* ± SD	p
A	10	0,196 ± 0,051	
B	10	0,154 ± 0,019	0,045*

Table 5. Liquid Chromatography-Mass Spectrometer (LCMS) Test Results on Castor Oil Leaf Extract (Ricinus communis)

No	RT (min)	Base peak (m/z)	Molecul Weight (g/mol)	Molecular formula	Tentative identification	Group compound
1	0,38	86,29	381,0	$C_{28}H_{46}O$	Brassicasterol	Fatty acid
2	0,74	120,24	167,11	$C_7H_5NO_4$	Quinolinic acid	Carboxylic acid
3	1,67	165,3	166,13	$C_5H_{10}O_6$	Ribonic acid	Sugar acid
4	2,74	60,14	464,37	$C_{21}H_{20}O_{12}$	2'-Hydroxyisoorientin	Flavonoid glycoside
5	3,686	60,21	588,5	$C_{29}H_{30}O_{13}$	Kolaflavone	Flavonoid
6	3,89	287,4	701,93	$C_{25}H_{32}O_{13}$	Oleuropein glycoside	Secoiridoid glycoside
7	4,179	397,15	814,96	$C_{37}H_{34}O_{21}$	Isorhamnetin 3(7)-O-[2'- O-coumaroyl- glucuronopyranosyl-(1- 2)-O-glucuronopyranoside]	Tetrahydroxyflavone
8	4,38	60,21	929,7	$C_{30}H_{42}N_7O_{19}P_3S$	Caffeoyl CoA	Glycoside
9	5,42	60,14	426,7	$C_{30}H_{50}O$	Lupeol	Terpenoid
10	5,81	60,21	367,31	$C_{16}H_{17}NO_9$	Xanthurenate 8-O-beta-D-glucoside	Glycoside
11	6,6	274,6	362,24	$C_{21}H_{30}O_5$	Humolones / α-lupulic acid / α-bitter acid	Terpenoid

IV. Discussion

The inhibition formed indicates the absence of Candida albicans growth around the paper disk due to the antimicrobial effect of chlorhexidine which can significantly reduce the growth of microorganisms, especially Candida albicans. Chlorhexidine has a mechanism of action, namely by binding to the fungal cell surface through

ionic bonds, chlorhexidine also has a high degree of antimicrobial activity which when it binds to fungal cell membrane components causes changes in the integrity of the fungal cell wall. The change in the integrity of the cell wall causes the function of the cell membrane to disappear. In Rakhmatullah's 2018 study, it was found that chlorhexidine was effective in inhibiting the growth of Candida albicans by producing a larger inhibition zone than the starfruit leaf extract group.[8]

Groups B and C also produced a larger inhibition zone than group A. This was due to the anti-fungal content contained in the Jatropha castor leaf extract, namely flavonoids, saponins, tannins, and terpenoids. The mechanism of action of flavonoids against Candida albicans is to disrupt cell membranes by forming cell extract protein complexes and their cell walls undergo protein denaturation through hydrogen bonds in the fungal cell wall. The fungal cell wall has an important role in the survival of the fungus and its pathogenicity, which is a site of ion exchange and protein filtration, which is the metabolism and catabolism of complex nutrients.[9]

The mechanism of action of saponins is to reduce the tension of the sterol membrane which plays a role in the synthesis of Candida albicans cell walls so that their permeability increases. Increased permeability can result in the more concentrated intracellular fluid being pulled out which will then cause nutrients, metabolic substances, enzymes, and proteins in the cells to come out and the fungus to die. Tannin is one of the chemical compounds contained in Jatropha castor leaf extract which also has effectiveness as an anti-fungal. Tannins, which are complex compounds of polyphenols, have a mechanism of action, namely reacting with cell walls and being able to inhibit cell synthesis of chitin, which is a component of Candida albicans. Terpenoids are also compounds that have effectiveness as strong antifungals against various pathogens, especially Candida albicans. Terpenoids can inhibit fungal growth, either through the cytoplasmic membrane or interfere with the growth and development of Candida albicans spores, one of the mechanisms of action is to create non-specific membrane lesions on the Candida albicans cell wall.[10] This is in accordance with research conducted by Pulungan in 2017, the results obtained that turmeric leaf extract was effective in inhibiting the growth of Candida albicans at a concentration of 50% obtained an average inhibition zone diameter of 6.10 mm, a concentration of 60% obtained an average inhibition zone diameter of 7.47 mm.[11]

V. Conclusions

Based on the results of the LCMS test, the compounds contained in the castor oil plant leaf extract are Flavonoid, Saponin, Tannin, and ricinoleic acid compounds. Flavonoid compounds in the LCMS results are 2'-Hydroxyisoorientin and Kolaflavone. Saponin compounds include lupeol and α-lupulic acid. Tannin compounds in the LCMS results are Galactinol and Quinolic acid. 2. The diameter of the inhibition zone of phytochemical compounds in the castor oil plant leaf extract (Ricinus communis) as a denture disinfectant against Candida albicans is 13.09 ± 1.02 mm in chlorhexidine, 14.47 ± 1.249 mm in the 50% concentration of castor oil plant leaf extract (Ricinus communis). 3. Based on the values obtained in this study, the 50% concentration of castor oil plant leaf extract has a larger inhibition zone against Candida albicans than chlorhexidine.

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