Running title: Gel Containing Ketepeng Cina Extract as An Anti Candida

# Formulation and Evaluation of Natural Anti Candida albicans Gel Containing Ketepeng Cina (*Cassia alata* L.) Leaves Extract

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# Novelty statement

Research related to gel formulation with the active ingredient Cassia alata extract with anti-candida albicans activity is an innovation in the development of a new anti-fungus that contains active plant extract in an effort to overcome resistance and existing antifungal side effects.

# Abstract

*Cassia alata* L. or locally known as gelenggang (Malaysia) or ketepeng cina (Indonesia) has been used as a traditional medicine to treat various diseases, especially skin diseases. Many studies have found that *C. alata* extract showed promising antimicrobial activity against skin-infecting microorganisms such as *Candida albicans*. Using extract as it is was found to be ineffective considering the instability of the extract after skin application. This study aimed to evaluate the stability and the antifungal activity of *C. alata* leaves extract gel against *C. albicans* with Carbopol 940 as the gelling agent. The microdilution method was used to determine the Minimum Fungicidal Concentration (MFC) value of *C. alata* leaves ethanol extract against *C. albicans*. *C. alata* leaves extract with concentrations of 0,31%; 0,62%; and 1,25% were incorporated into gel formulation and evaluated for its physical parameters and stability. Antifungal activity of the gel formulations was carried out by the well diffusion method. The MFC value was found at the concentration of 0,16% where no growth of *C. albicans* had been found. Gel formulations of *C. alata* L. leaves extract exhibit good physical characteristics and the results of the stability test showed unstable formulations in viscosity and spreadability measurement. Antifungal activity of *C. alata* extract gel with the concentrations of 0,31%; 0,62%; and 1,25% showed an inhibition zone of 12 mm; 14,7 mm; and 20,7 mm respectively. A gel containing 0,31% of the extract showed similar antimicrobial activity to ketoconazole 2% positive control. This study showed that *C. alata* gel had the potential as an alternative herbal topical medicine to treat skin infections caused by *C. albicans*. © 2023 Friends Science Publishers

**Keywords:***Cassia alata; Candida albicans*; herbal gel

# Introduction

Under normal conditions, *Candida albicans* is considered a normal flora on human skin or mucous membranes. However, under certain conditions, eg. during immunodeficiency, it can become infectious leading to a skin infection (Prastiyanto *et al*., 2021). One of the traditional medicinal plants that are often used to treat skin diseases is ketepeng cina (*Cassia alata* Linn) (Rumayar *et al.*, 2020). Previous research for this claim has been reported, the ethanol extract of *C. alata* leaves with a concentration of 50 mg/mL (5%) had an inhibition zone of 26.20 mm and a Minimum Inhibitory Concentration (MIC) value of 5.60 mg/mL against *C. albicans*. The result showed, the inhibition zone is larger than ketoconazole 200 mg/mL (2%; 24 mm) (Timothy *et al*., 2012).

To facilitate the use of *C. alata* leaves as a topical treatment, a formulation of the plant extract is needed. For this purpose, a water-based gel is chosen because the ethanol extract of *C. alata* leaves has poor solubility in water but has a higher water-soluble essence content than the ethanol-soluble extract (Angelina *et al*., 2021). Furthermore, compared to other semisolid dosage forms, gels are easier to apply and have a better release of active substances as well as percutaneous absorption. A gel dosage form of *C. alata* leaves extract has been reported that methanol extract in the hydrogel preparation shows better efficacy than the Daktarin® control against *C. albicans*, with a MIC value of 120 μg/mL vs 160 μg/ml (Iraqui *et al*., 2019).

Therefore, testing the antifungal activity of ethanol extract of *C. alata* leaves in a gel formulation against the fungus *C. albicans*, as well as testing the physical properties and stability of the gel formulation seems warranted.

**Materials and methods**

**Material and Apparatus**

**Materials**: Ehanol extract of C. alata leaves was prepared in November 2021 at Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN) as previously reported by Angelina *et al.* 2021 . *Candida albicans* INAcc obtained from Lembaga Ilmu Pengetahuan Indonesia (LIPI). Ingredients for gel formulation are carbopol 940, Na-CMC, propylene glycol, glycerin, Na benzoate, DMDM Hydantoin, and triethanolamine. The media used to test the antifungal activity were Sabouraud Dextrose Broth (Himedia, Japan), Bacteriology Agar, aquadest, alcohol 70%, McFarland's solution 0.5 (Himedia, Japan), and the active ingredient Ketoconazole (Aarti Drugs Limited, India).

**Apparatus:** Apparatus used were ruler, mortar and pestle, sieves, loop needles, tweezers; magnetic stirrer, disc paper, bunsen, petri dish (Citotest, China), water bath (GFL-1031, Germany), pH meter (Laqua PC-1100S Horiba, Japan), viscometer (Rotavisc me-vi IKA, China), beaker objects, Erlenmeyer glass and measuring cup (IWAKI, Japan), test tube, autoclave (Sterilizer SM 310 Yamato, Japan), climatic chamber (Memmert, Germany), incubator (JP-Selecta INCUDIGIT-TFT, Barselona), refrigerator ( LG MEZ64100092, South Korea), analytical balance (Mettler Toledo, Switzerland), micropipette (Eppendorf, Germany), Bio Safety Cabinet (LabTech United States), vortex (Velp, Italy), overhead stirrer (Labortechnik power control-visc IKA, China), 96-well Microplate round-bottom (IWAKI, Japan), hotplate (IKA, China).

# Gel formulation

Organoleptic properties of Carbopol and Na-CMC gel bases were compared (Table 1 and Table 2). The gel base with better properties was selected and then formulated with various concentrations of *C. alata* extract (Table 3). The concentrations of the extract were obtained after an antifungal activity test against *C. albicans*. Finally, 4 gel formulas consisting of 4 extract concentrations (0%, 0.31%; 0.62%; 1.25%) were tested for antifungal activity and physical properties, ie. organoleptic, pH, viscosity, rheology, homogeneity, and spreadability test. Accelerated stability tests to examine the physical properties were also performed.

# Physical properties test of gel formulation

Physical properties tests, ie. organoleptic, pH, viscosity, rheology, homogeneity, and spreadability test, were performed on freshly made gel and the gel underwent stability tests.

**Organoleptic**: Gel, was observed in shape, odor, and color.

**pH**: the pH was measured 3 times after diluting 1 g gel in 4 ml aquadest (Rahmawati & Setiawan, 2019).

**Viscosity and Rheology:** Viscosity test was performed on 100 g gel sample. 3 times for each sample at velocities 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, and 200 rpm. The viscosity at each rpm is measured at the 15th spindle rotation. The spindle used is the spindle which shows the % torque value in the range of 10-100% (Rahmawati & Setiawan, 2019). The relationship between velocity vs viscosity was depicted in a curve. Rheological properties were determined by measuring the % torque of the gel at the same velocity point when the velocity value increases and decreases. The relationship between velocity when it rises and falls vs % torque was depicted in a curve.

**Homogeneity:** Gel samples at the top, middle and bottom parts were placed on an object glass. Another object glass was placed on top of the gel and was squeezed until it spread evenly on the glass surface. Homogeneity is indicated by the absence of coarse grains in the sample (Rahmawati & Setiawan, 2019).

**Spreadability**: 1 g gel sample was placed on laminating film above graph paper. Another laminating film was placed on top of the gel and the spreading gel area was calculated. Then, a 19 g weight (consisting of a 10 mL beaker glass and a calibration weight) was placed on top of it and the area was calculated after 1 min stands. The spreading gel area formed after another series of weights was also calculated, ie. 39 g, 59 g, 79 g dan 99 g. The test was performed in triplicate. The relationship between weight and area was depicted in a curve (Rahmawati & Setiawan, 2019).

**Accelerated stability:** Gel sample was stored in airtight and opaque containers under conditions designed to accelerate changes that would normally occur under normal conditions. The accelerated stability test was carried out at a temperature of 40 ± 2°C with a relative humidity of 75% ± 5% for 4 weeks in a climatic chamber (Ponnusamy *et al*. 2010). Then, the physical properties test were performed on the 2nd and 4th weeks (Sayuti, 2015).

# Statistical analysis

The data obtained in the accelerated stability test were analyzed statistically using SPSS with modification (Sayuti, 2015). Repeated measure one-way variance (ANOVA) was used for normal and homogeneous data. Friedman analysis was used for non-normal data. p-values <.05 were considered to be statistically significant, ie. the gel is unstable.

# Antifungal activity test against *C.albicans*

**Preparation of C. albicans inoculums:** *C. albicans* fungus was cultured by scraping the fungus using an inoculating loop on Sabouraud Dextrose Agar and incubated at 30°C for 2 days. Using an inoculating loop, the culture of the *C. albicans* fungus was then suspended in 0.9% NaCl solution and homogenized using a vortex. Turbidity was compared with 0.5 McFarland solution which is equivalent to 1 x 108 CFU/mL (Ohikhena *et al.* 2017).

**Ethanol extract of C. alata leaves:**A stock solution of 100,000 μg/mL (10%) of ethanol extract of *C. alata* leaves was prepared by dissolving 1 g of the extract in 10 ml of dimethylsulphoxide: NaCl 0.9% (1:9) in a glass vial (Ergashev, 2021). In a 96-well plate (Figure 1), 100 μl of SDB medium was added to each well of rows A, B, C, and E. Using a multichannel pipette, 100 μl of stock extract solution was added to well 1A, 1B, 1C, and 1E and then homogenized five times. Serial dilution of this mixture was performed in the next well until wells 10A, 10B, 10C, and 0E. Next, 100 μL of *C. albicans* inoculum solution was added and homogenized in each well of rows A, B, and C, while 0.9% NaCl solution was added and homogenized in row E. The concentrations of *C. alata* extract obtained in columns 1 to 10 were 2.5%; 1.25%; 0.62%; 0.31%; 0.16%; 0.08%; 0.04%; 0.02%; 0.01%; 0.005%, respectively. Wells 11A, 11B, and 11C were used as negative controls and filled only with SDB media and *C. albicans* inoculum solution. Wells 1E to 11E was used as a comparison of the turbidity color of extracts without microbes. Column 12 was used as a positive control containing 2% ketoconazole. After incubation at 30°C for 2 days, the turbidity of the mixture and the presence of fungal deposits on the bottom of the wells were observed. MIC was depicted by a clear mixture without fungal precipitate. MBC value was determined by inoculating a series of extract solutions from the well plate (starting from MIC to the highest concentration) on SDA media in a petri dish. The Petri dishes were then incubated at 30°C for 2 days. MBC was depicted by an agar medium without fungal colonies (Felipe *et al.* 2013).

**Gel-containing ethanol extract of C. alata leaves:** A petri dish was divided into several quadrants then SDA media was added and allowed to harden. After that, 100 μl of *C. albicans* inoculum suspension was spread evenly using an L rod on the surface of the medium and let dry for 5 minutes. A well was made in each quadrant using a cork borer with a diameter of 6 mm. Then, the well was filled with the test samples, ie. formulation 1-4, carbopol gel formulation containing ketoconazole, and ethanol extract of *C. alata*. Finally, the petri dish was incubated at 30°C for 48 hours. Antifungal activity was determined by measuring the clear area formed around the wells. The clear area is the inhibition zone of the test samples against *C. albicans* (Felipe *et al.* 2013).

# Results and Discussion

**Basis gel comparison**

Water-based preparations were chosen because *C. alata* ethanol extract is polar . Besides, it gives a cold sensation, dries easily, forms a film that is easily washed off, and easily releases the active ingredients (Rahmawati & Setiawan, 2019; Sayuti, 2015) . Gel formula consists of active ingredients, gelling agents, and other additives such as humectants and preservatives. It is the gelling agent that determines the physical properties, stability, and ability to release the active ingredients to the skin (Sayuti, 2015).. The gelling agents commonly used are carbopol and sodium carboxymethylcellulose (Na-CMC). Carbopol is a hydrophilic polymer with a polyacrylic acid structure and produces a transparent, bioadhesive gel. It produces a good viscosity value of 4,000-11,000 cP at a concentration of 0.5%. Sodium carboxymethylcellulose at a concentration of 3 to 6% is often used because it has a good viscosity and is neutral (Sayuti, 2015).

The gel base was selected by comparing the organoleptic of 100 grams of gel preparation, ie. thickness, stickiness, and texture. As seen in table 4, carbopol base was chosen because it has clear color, is less sticky, spreads more easily, gives a cold sensation, dries faster and is easily washed off with water. The Na-CMC gel base has a yellowish color, is not clear, more sticky, thicker and less spreadable. Because the optimum concentration for Na-CMC gel base is 3-6%, reducing the concentration will only produce a liquid state (Setiawati *et al*. 2021). Therefore, carbopol 940 was chosen as the base gel in this study. Other advantages of carbopol as a gelling agent are (1) it only requires water at room temperature during gel development unlike Na-CMC which requires hot water, (2) it provides high viscosity at low concentrations, (3) it has a wide viscosity range, (4) it is compatible with many active ingredients, (5) it has good stability at different temperatures and (6) it has organoleptic characteristics that are well accepted by users (Setiawati *et al*. 2021).

**Antifungal activity of *C. alata* leaf ethanol extract against *C. albicans***

Based on Timothy et al, *C. alata* leaves ethanol extract at a concentration of 2.5% showed inhibition of 19 mm against *C. albicans*. Therefore, we used 2.5% as the highest extract concentration (Rumayar e*t al*., 2020). Our data showed the MIC value of the *C. alata* extract against *C. albicans* was obtained at a concentration of 0.04% (Table 5). From this concentration upwards, the media was not cloudy and there was no sediment at the bottom of the well indicating no fungal growth. Meanwhile, the MBC value of the extract was obtained at a concentration of 0.16% (Table 4) where no growth of colonies on the media was observed. This is almost similar to Owoyale et al., In their study, the ethanol extract of *C. alata* leaves has a MIC value of 0.08% against *C. albicans*. Another study by Ponnusamy et al reported an MBC value of the ethanol extract of *C. alata* leaves against *C. albicans* was 0.2% ppm (Ponnusamy *et al*. 2010).

The LC-MS study revealed several chemical compounds in the extract, such as emodin, kaempferol, kaempferol-3,7-diglucoside, and kaempferol-3-O-β-D -glucopyranose (Angelina *et al*., 2021). According to Hennebelle et al, kaempferol and aloe-emodin are the most active anthraquinone derivatives that possess antimicrobial activity (Hennebelle *et al*., 2009). The mechanism of action of kaempferol as an antifungal agent is by inhibiting the synthesis of nucleic acids in fungal cells. According to Sule et al, anthraquinone compounds have great potential as antimicrobials. Targets in fungal cells are adhesins on the cell surface, polypeptides on the cell wall, and enzymes bound to the cell membrane (Sule *et al.* 2010).

**Carbopol gel base formulation with *C. alata* extract as the active ingredient**

The gel base formulation consists of carbopol 940 as a gelling agent, propylene glycol as a humectant, DMDM hydantoin as a preservative, TEA as a wetting agent, and distilled water as a carrier. Carbopol is a high molecular weight synthetic polymer of acrylic acid that is crosslinked with allyl sucrose or allyl ether of pentaerythritol. Because of high carboxylic acid groups (56% to 68%), it is a weak acid with a pH value of 2.8 to 3.3 and easily converts to its salt form (Ergashev, 2021). After dispersing carbopol in water , the solution was then neutralized until pH 6-11 by adding an alkalinizing agent, such as triethanolamine (TEA) that results in water absorption leading to a stronger microgel bonds formation . Carbopol 940 was chosen because of economical reasons and the concentration used is 1% because the range for a good gel formation is 0.5% -2% . Humectants, such as propylene glycol, are an additive that maintains the stability of the gel by absorbing moisture and reducing water evaporation . Triethanolamine (TEA) is an alkalizing agent that neutralizes the carboxylic groups of the carbopol polymer to form a gel base. TEA ionizes Carbopol and generates a negative charge along the polymer structure resulting in electrostatic repulsion and leading to a dense gel mass formation (Ergashev, 2021). The high water content in gel preparation can attract microbes which may lead to physical and chemical changes in the gel, eg. changes in color, odor, and pH. Therefore, DMDM hydantoin was used as a preservative because of its broad antimicrobial spectrum, water solubility, and stability over a wide pH and temperature range. A concentration of 0.6% was chosen because it is the maximum concentration of DMDM Hydantoin in cosmetics allowed by the Indonesian FDA is 0.6% (Number 23 of 2019). Based on the antifungal activity of the ethanol extract (Table 4), 4 gel formulations of the extract, ie. 0%, 0.31%, 0.62%, and 1.25%, were tested for its physical properties at week 0 as well as at week 2 and 4 (Table 6).

**Organoleptic Test**

Organoleptic examination was carried out by observing the physical appearance of the gel preparation such as the color, smell, and texture/shape of the preparation (Ohikhena *et al.* 2017). All four formulas had different colors due to the different extract content because the higher the concentration of the extract, the darker the color. The texture is similar in all four formulas because of the similar concentration of the gelling agent. The accelerated stability test results for 4 weeks showed no change in color, odor, texture, and separation of the base from the extract as well, indicating stability.

**pH Determination Test**

The pH value was checked to prevent skin irritation caused by an unsafe pH range (beyond 4.5-5). In these formulations, the extract is acidic while TEA is alkaline. The higher the concentration of the extract, the higher the amount of TEA needed. The accelerated stability test results for 4 weeks showed that the pH value was not significantly different, indicating stability.

**Viscosity Test and Rheology**

The viscosity of the gel affects the spreadability. After plotting the measured viscosity at the y-axis and the spindle speed on the x-axis, it was observed that the viscosity of the *C. alata* extract gel preparation decreased with increasing spindle shear speed. The difference in viscosity for each formula was due to the decrease and increase in shear speed, indicating a non-Newtonian liquid. Formula 1 decreased from 90,800 Cp to 7680 cP, formula 2 from 72,000 cP to 3844 cP, formula 3 decreased from 50,267 cP to 4593 cP, and formula 4 experienced a decrease in viscosity from 36,533 cP to 3327 cP.

pH value may affect viscosity because the higher the concentration of the extract, the more TEA was needed, and the higher the pH value, leading to increased viscosity (Ohikhena *et al.* 2017). This is inconsistent with our results where the viscosity of the *C. alata* gel preparation is inversely proportional to the increase in pH, resulting in a decreased viscosity as the pH value increases. This shows that the *C. alata* extract affects the viscosity of the preparation where the more the extract, the lower the viscosity. This is supported by the results of the blank preparation viscosity value (F1), where a lower pH value compared to 1.25% preparation (F4), a higher viscosity value is obtained.

The rheological properties of the gel are strongly influenced by the concentration and structure formed by the polymer. It may affect how long the gel remains at the application site and the speed of drug release (Ohikhena *et al.* 2017) and also affect the selection of equipment during the production process. It is described using a curve with spindle speed (rpm) as the x-axis and %torque as the y-axis. Our data showed that gel preparation of *C. alata* has pseudoplastic flow properties where the curve starts at or approaches the origin, namely point (0,0) at low shear speeds (Ohikhena *et al.* 2017). This is consistent with a study conducted by Islam *et al.* (2004) where carbopol gel showed the same flow properties at several temperatures. Pseudoplastic preparations immediately flow when a shear stress is applied and their viscosity decrease when the shear stress increases. The narrowness of the up and down curves indicates that the flow properties of the carbopol gel are not affected by time. This indicates that it does not take time to return to its original structure when given a certain shear rate .

The accelerated stability test results for 4 weeks showed that stable preparations were only found in gel blank preparations (F1, p> 0.05). *C. alata* gel preparations showed significant changes in the viscosity caused by temperature and humidity changes (p <0.05) in which the higher the concentration of the extract, the greater the decreased viscosity. The biggest decrease in viscosity was found in the 1.25% preparation where the viscosity greatly decreased at low speed. Changes in viscosity can be associated with changes in the pH where the viscosity value decreases as the pH value decreases. This is due to the nature of carbopol as a gel base which depends on pH changes. For further study, to minimize the effect of pH changes (1) a buffer can be added (2) a gel base that is inert to pH changes can be used.

**Homogeneity Test**

Homogeneity test is carried out by observing the physical properties of a preparation at room temperature. A preparation is homogeneous when no granules, coarse particles, lumps, and uneven color were observed. The results of the homogeneity test showed that the four gel preparations had good homogeneity. This shows that the gelling agent expands perfectly and mixes well with the active ingredients and other additives. The results of the accelerated stability test for 4 weeks with a temperature of 40ºC and 75% RH showed similar results indicating stability.

**Spreadability Test**

Spreadability test was carried out to see the ability of the preparations, including the active ingredients, to spread when applied to the skin. It reflects the comfort level of the user in which the greater the surface area, the easier the preparation is to be smeared. The data showed that the higher the concentration of the extract, the wider the spreadability of the preparation, which is in line with another study (Ohikhena *et al.* 2017). The spreadability area is also related to the viscosity of the preparation where the lower the viscosity, the easier it spreads or flows, and the bigger the spreadability area. A good spreadability of semisolid is with a diameter of 3-5 cm or an area of 7.605-19.625 cm2 (Ohikhena *et al.* 2017).. This indicates that the four formulas have good dispersing properties. In the accelerated stability test results for 4 weeks, the spreadability at a load of 59 grams showed a significant difference, indicating instability. Based on the curve of the spreadability of the gel, there was a decrease in the spreadability of the gel after two and four weeks. The area of spreadability is inversely proportional to the viscosity. Therefore, the area of spreadability increases if the viscosity decrease. This is not following the scatter power where the area of spreadability decrease when the viscosity decrease. This difference may be caused by errors during measurement such as the spreadability test not immediately carried out after weighing. The temperature may affect spreadability because the lower the temperature, the higher the viscosity, and the smaller the spreadability area. Furthermore, water evaporation may lead to increased viscosity. Because of this error, the data is considered invalid.

**Antifungal Activity Test of Chinese Ketepeng Leaf Ethanol Extract Gel**

Antifungal test of *C. alata* gel at concentrations of 0.31%; 0.62%; 1.25% against *C. albicans* shows respective inhibition zones of 13.3 ± 1.15; 16.0 ± 2.00; 18.0 ± 1.00 mm (Table 7). A concentration of 0 % or negative control does not show any inhibition zone while the positive control (2% Ketoconazole gel) shows the inhibition zone with a diameter of 12.0 ± 0 mm. The higher the extract concentration, the greater the inhibition zone.

*C. alata* extract at a concentration of 0.31% showed antifungal activity equivalent to 2% ketoconazole gel. This is slightly different from another study that reported 5% *C. alata* ethanol extract has an inhibition zone equivalent to 2% ketoconazole (Timothy *et al.*, 2012). This difference may be caused by (1) different geographical locations where the plants grow leading to the different chemical content of the extract, (2) different methods of extraction, drying process, and concentration of solvents. Of note, the gel preparation containing 0.31% extract produced a larger inhibition zone compared to the 2% ketoconazole gel. All three preparations produced an inhibition zone with a diameter of 12-18 mm, indicating a strong inhibition against *C. albicans* (Timothy *et al.*, 2012)..

The 0.31% and 0.62% *C. alata* gel also showed a larger inhibition zone than *C. alata* extract at the same concentration. This is following a study conducted by Iraqui *et al.* (2019), where a gel preparation of 1% *C. alata* methanol extract had a significantly larger inhibition zone than *C. alata* methanol extract without gel preparation. This is because the extract solution, when inserted into the hole in the agar media, settles to the bottom of the petri dish so that the chemical compounds in the extract cannot diffuse evenly into the medium. Carbopol will cross-link with the extract solution thereby preventing the extract from precipitating and providing a better release of active ingredients compared to the extract alone (without gel).

# Conclusion

The ethanol extract of *C. alata* leaves showed fungistatic activity at a concentration of 0.039% and fungicide at a concentration of 0.16% against the fungus *C. albicans*. The gel preparation of ethanol extract of *C. alata* leaves is stable based on organoleptic, homogeneity, and pH tests. However, the viscosity and spreadability were unstable in each sampling at weeks 0, 2, and 4. Gel preparation of ethanol extract of *C. alata* leaves with a concentration of 0.31%; 0.62%; and 1.25% showed strong antifungal activity with an inhibition zone diameter of 13.3 ± 1.15 mm; 16.0 ± 2.00mm; and 18.0 ± 1.00 mm, respectively.

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**Table 1**: Gel candidate with Carbopol base

|  |  |
| --- | --- |
| Materials | Concentrations |
| Carbopol 940 | 1 |
| Propylene glycol (PPG) | 5 |
| DMDM Hydantoin | 0.6 |
| Aquadest | ad 100 |
| Triethanolamine (TEA) | q.s |

**Table 2**: Gel candidate with Na-CMC base

|  |  |
| --- | --- |
| Materials | Concentrations |
| Na CMC | 3 |
| Propylene glycol (PPG) | 15 |
| Glycerin | 10 |
| Sodium Benzoate | 0.25 |
| Aquadest | ad 100 |

**Table 3**: Gel formulation of ethanol extract of *C. alata* L. leaves using Carbopol base

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Materials | Formula 1 (%) | Formula 2 (%) | Formula 3 (%) | Formula 4 (%) |
| Ethanol extract of *C. alata* L. leaves | 0 | 0.31 | 0.62 | 1.25 |
| Carbopol 940 | 1 | 1 | 1 | 1 |
| Propylene glycol (PPG) | 5 | 5 | 5 | 5 |
| DMDM Hydantoin | 0.6 | 0.6 | 0.6 | 0.6 |
| Aquadest | 93.4 | 93.09 | 92.77 | 92.15 |
| Triethanolamine (TEA) | 2.5 | 2.5 | 2.6 | 2.75 |

**Table 4**: Comparison of carbopol and na-cmc gel bases

|  |  |  |
| --- | --- | --- |
| Evaluation | Carbopol 940 | Na-CMC |
| Color | Clear/transparent | Yellowish white |
| Texture | Gentle; not sticky; thick | Gentle; sticky; very thick |
| Sensation | Cold | Not cold |
| Spreadability | Easy to spread | Slightly difficult to spread |

**Table 5**: MIC and MBC of *C. alata* extract against *C. albicans* based on figure 1.

|  |  |  |
| --- | --- | --- |
| Test Samples | MIC | MBC |
| 2.5 % *C. alata* extract | - | - |
| 1.25 % *C. alata* extract | - | - |
| 0.62 % *C. alata* extract | - | - |
| 0.31 % *C. alata* extract | - | - |
| 0.16 % *C. alata* extract | - | - |
| 0.08 % *C. alata* extract | - | + |
| 0.04 % *C. alata* extract | - | + |
| 0.02 % *C. alata* extract | + | NA |
| 0.01 % *C. alata* extract | + | NA |
| 0.005 % *C. alata* extract | + | NA |
| 2% Ketoconazol | - | NA |
| Media + Inoculum | + | NA |

Note: (-) no fungal growth; (+) fungal growth observed.

**Table 6:** Physical properties of Carbopol gel base containing *C. alata* extract

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| F | W | Organoleptic | | | pH | Viscositya (cPs) | Rheology | Homogenity | Spreadabilityb  (cm2) |
| Color | Smell | Texture |
| F1 | W0 | Clear | Carbopol | Gentle; Thick | 4.74 ± 0.04 | 7680 | Pseudo-plastis | Homo-geneous  no coarse particles | 7.71 ± 0.29 |
|  | W2 | 4.75 ± 0.11 | 7678 | 6.91 ± 0.27 |
|  | W4 | 4.76 ± 0.03 | 7807 | 5.87 ± 0.49 |
|  | P |  |  |  | 0.574 | 0.651 |  |  | c |
| F2 | W0 | Yellow-ish-brown | extract | Gentle; Thick | 4.60 ± 0.01 | 3567 | Pseudo-plastis | Homo-geneous  no coarse particles | 10.95 ± 0.67 |
|  | W2 | 4.60 ± 0.02 | 4013 | 7.07 ± 0.47 |
|  | W4 | 4.70 ± 0.10 | 4980 | 8.22 ± 0.78 |
|  | P |  |  |  | 0.145 | 0.001 |  |  | c |
| F3 | W0 | Dark brown | extract | Gentle; Thick | 4.76 ± 0.10 | 4593 | Pseudo-plastis | Homo-geneous  no coarse particles | 10.94 ± 0.34 |
|  | W2 | 4.60 ± 0.06 | 3307 | 8.90 ± 0.62 |
|  | W4 | 4.54 ± 0.05 | 3193 | 6.93 ± 0.98 |
|  | P |  |  |  | 0.068 | 0.000 |  |  | c |
| F4 | W0 | Dark choco-late | extract | Gentle; Thick | 4.81 ± 0.03 | 3327 | Pseudo-plastis | Homo-geneous  no coarse particles | 12.78 ± 0.73 |
|  | W2 | 4.78 ± 0.09 | 2760 | 9.80 ± 0.32 |
|  | W4 | 4.70 ± 0.01 | 2533 | 9.08 ± 0.53 |
|  | P |  |  |  | 0.103 | 0.001 |  |  | c |

Note: aViscosity at 200 rpm, bSpreadability at 59 g. c Asymp. Sig. 0,05, Chi-Square 6000, df:2. W0: Week 0 (freshly made), W2,W4 : Week 2, Week 4 (during accelerated stability test). F1-F4: Gel formulations of *C. alata* extract F1 (0% extract), F2 (0.31% extract), F3 (0.62% extract), F4 (1.25% extract).

**Table 7**: Antifungal activity of *C. alata* gel and extract against *C. albicans*

|  |  |
| --- | --- |
| Test samples | Inhibition zone(mm) |
| F 1 | 0 |
| F 2 | 13.3 ± 1.15 |
| F 3 | 16.0 ± 2.00 |
| F 4 | 18.0 ± 1.00 |
| 0,31 % *C. alata* extract | 12.0 ± 0 |
| 0,62 % *C. alata* extract | 14.7 ± 0.58 |
| 1,25 *C. alata* extract | 18.0 ± 0 |
| 2% Ketoconazole gel | 12.0 ± 0 |
| 2% Ketoconazole solution | 11.0 ± 0 |

Note: F1-F4: Gel formulation C. alata extract 1 (0% extract), F2 (0.31% extract), F3 (0.62% extract), F4 (1.25% extract)

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