

Analysis of fungi and yeast contamination on cabe jamu simplicia (*Piper retrofractum*) in Madura Indonesia

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Abstract. Cabe jamu has health benefits, making it one of the herbal commodities with economic value. The demand of cabe jamu is not only for domestic market as a raw material for the pharmaceutical industry, but also as an export commodity. As an export commodity, cabe jamu must be able to meet the standard of destination country. One of the standard is related to product sanitation. Cabe jamu, like other herbal commodities, are mostly economic businesses carried out by small industries, with relatively low sanitation levels, and it caused Indonesian herbal commodities failed to enter the international market. In the period 2014-2016 there were 54 cases of rejection of Indonesian spice (*Myristica fragrans*) exports to the European Union, United States, and Japan, with a loss of Rp 7.61 billion, due to microorganism contamination. This study aims to obtain the types of fungi and yeast that contaminate cabe jamu. Dried cabe jamu were collected from four different locations in the Madura region. The results of the inoculation on Potato Dextrose Agar (PDA) media revealed the formation of colonies that were mixed with one another. The purification resulted colony cultures with 3 of them included in the fungi genus *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*, while the other 2 were yeast genus *Saccharomyces sp.* and *Candida sp.*

1 Introduction

Cabe jamu is a type of chili known in traditional medicine for its health benefits and is often used as a natural ingredient in herbal remedies. This plant also holds high economic value as a herbal commodity in both domestic and international markets. This cabe jamu, which is a native Indonesian spice, not only has health benefits but also be beneficial in terms of the economy. The price per kilogram of Cabe jamu reaches Rp. 80,000 – Rp. 100,000 [1]. Thus, the prospect of cabe jamu cultivation is very necessary, in addition to meet the needs of the traditional medicine industry in the form of herbs and other domestic needs, as well as export commodity.

Cabe jamu has been widely accepted both in developing and developed countries. As many as 80% of the population of developing countries and 65% of the population of developed countries have used herbal medicine. The use of herbal medicine is currently increasing, both in developing countries and in developed countries (WHO, 2002) in [2]. According to a statement from the Minister of Trade, in the period January - September 2020, the export value of Indonesian herbal or biopharmaceuticals increased by 14.08% or worth 9.64 million United States (US) dollars compared to the same period in 2019. Indonesia's biopharmaceutical export destination countries include

India (62.30%), Singapore (6.15%), Japan (5.08%), Malaysia (3.75%), and Vietnam (3.17%). This data shows the huge potential of the Indonesian herbal medicine market on a global scale [3]. The world's need for cabe jamu alone is around 6 million tons, and Indonesia can only meet one-third of it. Several countries as export destinations for cabe jamu include Singapore, Malaysia, China, the Middle East, Europe, and America [1].

The use of traditional medicine to support the quality of health requires a balanced effort to improve the quality. Therefore, to protect consumers from the consequences of using traditional medicines that do not meet the requirements, traditional medicines must be tested for quality, safety from contamination, and usefulness so that they can be circulated in Indonesia and exported abroad [4]. According to Hafif (2021), in Indonesia in 2014 - 2016 there were 54 cases of rejection of spices in the form of nutmeg commodities which were exported to the European Union, the United States, and Japan, with a loss value of Rp. 7.61 billions [5]. One of the reasons for the rejection of Indonesian nutmeg products is the mycotoxin content which the frequency of rejection reaches 40 cases. As for the case of mold contamination in feed ingredients in the United States, the loss was US\$ 1.60 billion/year, and in several countries in Asia in 1999 it reached US\$ 400 million/year [6]. Fungi are contaminants that are often

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found in herbal medicines, where 50% of the 91 samples of medicinal plants in Brazil were contaminated with fungi [7]. According to Guchi (2015), it is estimated that a quarter of the world's food crops are contaminated with mycotoxins [8]. This incident in addition to disturbing health also resulted in a fairly high economic loss. Efforts can be made to avoid contamination by microorganisms, namely by applying good hygiene and sanitation [9].

Research on identifying microbial contamination is an important thing to do because the most preferred herbal preparations in the local market are in the form of liquids, capsules, and powders which are then brewed [10]. The way of presenting herbal medicine by brewing cannot kill microorganisms, because at hot temperatures microorganisms have a system to maintain their life [11]. So far, the use of cabe jamu has primarily focused on its application as a traditional herbal remedy and has not yet explored its relation to fungi and yeast. Therefore, this research is essential to identify gaps in current sanitation practices and propose improvements that reduce contamination risks, ultimately contributing to healthier, safer conditions for all.

2 Materials and method

Research was carried out at the Laboratory of Quality Analysis of Agricultural Industrial Technology, Faculty of Agriculture, Trunojoyo University, Madura. The study was conducted from July 2022 – December 2022. Sampling was carried out in several areas on the island of Madura. The materials used in the study included dried cabe jamu obtained from the Madura island, Potato Dextrose Agar (PDA), aquadest, alcohol, spirit, aluminum foil, label paper, brown cover paper, tissue, and cotton.

2.1 Preparation of culture media

Potato Dextrose Agar (PDA) in the form of a cup medium is the medium used in the study. PDA media was made by mixing 8 grams of instant PDA powder and 200 mL of sterile distilled water in an erlenmeyer. The next step is the Erlenmeyer containing the mixture is placed on a hot plate until it boils and is clear while stirring slowly. The clear media was then given a cotton swab in the mouth of the Erlenmeyer and then coated with aluminum foil.

2.2 Inoculation

Inoculation process using the modified method by Intan [12]. Inoculation was done by direct planting of dried cabe jamu fruit tissue culture. Direct inoculation was carried out by taking each sample of herbal chili tissue with a sterile ose needle, then transferring it to the culture medium using the zig-zag streak. After inoculation, each sample was labeled with a description on the lid of the petri dish. The media that had been planted and labeled were then wrapped in brown paper and then incubated by storing them in an incubator for

about 2 days at room temperature maintained at 37 C. Inoculation was carried out several times to obtain one type of colony.

2.3 Identification of fungi and yeast

Fungi and yeast that had been incubated for about 2 days were then identified macroscopically and microscopically. Macroscopic identification is by observing the development of diameter, top and bottom surface color, surface type, and the shape of fungi and yeast. Microscopic identification was carried out by growing fungi and yeast on slide culture. Microscopic characteristics can be done by observing the presence or absence of spores or conidia, rhizoids, hyphae type, spore shape, and conidia using a microscope. The microscopic characteristics of yeast observed were the form of conidiophores, conidia, and hyphae or pseudohyphae. The results of observations both macroscopic and microscopic identification are then matched with the key book identification or other relevant literature studies. The complete research stages are shown in Figure1 below.

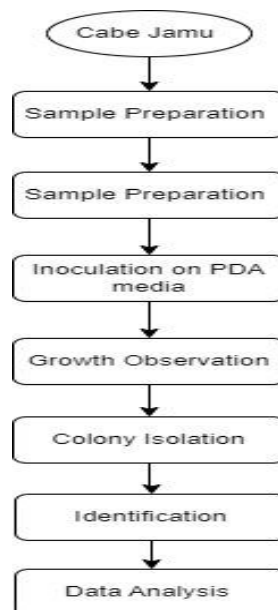


Fig. 1. Research stages.

3 Results and duscussion

Results of inoculation and identification of fungi based on their morphological characteristics are presented in Figure 2.

Identification of fungal colonies was carried out macroscopically and microscopically. Identification macroscopically using a tool in the form of a camera, meanwhile microscopically using a light microscope connected to the OptiLab Viewer application. The following is the result of the identification of fungi both macroscopically and microscopically (Figure 3).

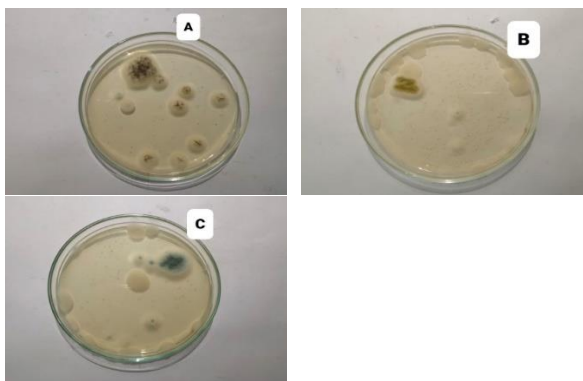


Fig. 2. Fungi colonies of *cabe jamu*.

3.1 Code A colony

Macroscopically, the fungal culture has the characteristics of a colony with color blackish brown surface with white border, flat surface, flat edge with a texture resembling cotton. Fungi with these characteristics classified under the species *Aspergillus niger*. Descriptive analysis based on research by Wulandari *et al.* (2016), which stated *Aspergillus species niger* macroscopically as shown in the picture has the characteristics of a colony edge flat, soft texture and round shape, and black colonies [13]. Characteristic- The characteristics that show the fungus in the picture belong to the *Aspergillus species niger* is also supported by the results of a study by Abdulrahman *et al.* (2019), that *Aspergillus niger* is found to be velvety and has a dark brown color to black [14].

The results of microscopic observations obtained the characteristics of fungi with round conidia and vesicles, thick elongated conidiophores, as well as have septate conidiophores (Figure 3). Fungi with these characteristics are classified in *Aspergillus niger* species. Descriptive analysis adapted to research by Rahman and Zakaria (2020), results of microscopic observations the shape of the fungus is obtained according to the characteristics of the species *Aspergillus niger* namely have dark brown to black conidia heads, conidiophores smooth-walled with a thick and long shape, and there are vesicles large round shape covered by filialids on the surface [15]. Based Irma's research in 2015 in Paramita (2021), microscopically on *Aspergillus niger* contains conidia, vesicles, and hyphae and septate conidiophores [16].



Fig. 3. Microscopy of Code A Colonies (1) *Conidia*, (2) *Vesicles*, (3) *Conidiophores*, (4) *Conidiophores Septa*.

3.2 Code B colony

Colonies of code B fungi have macroscopic characteristics yellowish green surface with white

border flat surface, flat edges, and resembling cotton. Colonies with features These are classified under the fungus species *Aspergillus flavus*. Descriptive analysis based on research by Abdulrahman *et al.* (2019), which mentions The color of *Aspergillus flavus* colonies is yellowish green with a white tint in the edge area [14]. According to Octavia and Wantini (2017), macroscopically filamentous *Aspergillus flavus* colonies appear, texture like cotton and velvet, and a white green surface with yellow areas [17].

Microscopic identification obtained the characteristics of a fungus colony It has yellow conidia, branched hyphae, round vesicles and conidiophores long shape (Figure 4). Based on its characteristics, fungal colonies are included in species *Aspergillus flavus*. Descriptive analysis adapted to the results of research by Kurniawati *et al.* (2021), *Aspergillus flavus* has conidia and vesicles round to semi-spherical, conidia green, and relative conidiophores rough [18]. According to Fitria and Setiawati (2020), microscopically on *Aspergillus flavus* contains branching mycelia, long conidiophores and conidia yellow to green in color with a chain-like shape [19].

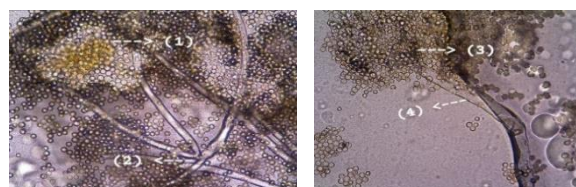


Fig. 4. Microscopy of Code B Colonies (1) *Conidia*, (2) *Branching Hyphae*, (3) *Vesicles*, (4) *Conidiophore*.

3.3 Code C colony

The characteristics of the fungal culture are macroscopically visible on the surface of the colony dark green slightly blue-gray with a white border, type flat surface, flat edge, and cotton-like texture. Based on the identification results that have been carried out, the fungal colonies in the image above are included in the species *Aspergillus fumigatus*. Descriptive analysis based on research by Gandhi *et al.* (2019), stated that the species *Aspergillus fumigatus* identified macroscopically marked with green to green color old on the surface of the colony and on the edge is white, the edge of the colony flat, and has a smooth colonial surface with a velvety texture [20]. The results of research by Lestari *et al.* (2019) also confirmed that colonies *Apergillus fumigatus* is greenish in color [21].

The results of microscopic observation of code C colonies obtained characteristics fungi with conidia and vesicles are spherical, and conidiophores have a shorter shape (Figure 5). Fungi with these characteristics are classified in genus *Aspergillus fumigatus*. Descriptive analysis adapted to research by Prasetyaningsih *et al.* (2015), the results of direct observation microscopically, the shape of the fungus is obtained according to the characteristics of the *Aspergillus* genus *fumigatus* which has round to semi-spherical conidia, smooth-walled conidiophores and short, and there are vesicles that have

mace shape [22]. Based on the results of research by Urip et al. (2021), microscopically *Aspergillus fumigatus* is characterized by columnar-shaped conidia, not hyphae septate, club-shaped vesicles, smooth conidiophore walls, and conidiospores attached to the ends of the conidia [23].

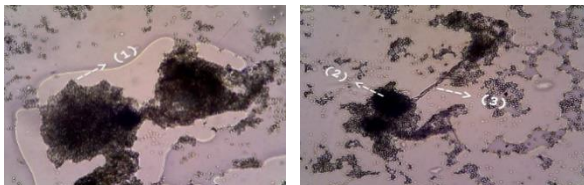


Fig. 5. Colony Microscopy Code C (1) Conidia, (2) Vesicles, (3) Conidiophores.

Results of inoculation and identification of yeast based on their morphological characteristics are presented in Figure 6 below.

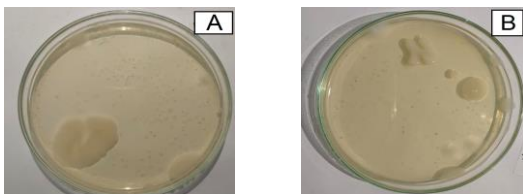


Fig. 6. Yeast colonies of cabe jamu.

Yeast isolate A has an irregular colony shape with a butter-like texture, cream-colored colonies with raised elevations and filament-like edges. According to Citra (2019), the genus *Saccharomyces* has colony growth characterized by light cream to dull beige colonies with a smooth surface and raised elevations and edges in the form of filaments [24]. Based on the microscopic morphological similarity of yeast colony growth, isolate A has a similar shape to the genus *Saccharomyces*.

Yeast isolate B has a round shape textured like butter. Colonies are creamy white, convex elevation and flat margins. According to research conducted by Indrayati et al. (2018) after identifying the genus *Candida*, it has the characteristics of colonies that are round, yellowish white in color, smell sour like the aroma of tape, colonies are shaped like yeast, the surface of the colonies is wet, smooth, smooth and convex [25]. Based on the macroscopic morphological similarity of yeast colony growth, isolate B has a similar shape with the genus *Candida*.

3.4 Code A colony

Based on the microscopic morphology of the yeast, isolate A measures $\pm 5\mu\text{m}$, has an ovoid cell shape, carries out vegetative reproduction by multilateral budding, so that based on its microscopic morphology, this yeast isolate has a similar shape to the genus *Saccharomyces* (Figure 7). This microscopic morphology is the same as the morphology of the genus *Saccharomyces* in a study conducted by Citra (2019) [24]. In addition, another feature of the genus *Saccharomyces* is that according to Widiastutik and

Alami (2014) it does not form hyphae but allows the formation of pseudohyphae and reproduces sexually with ascospores (1-4 per ascus) [26].

Another characteristic of *Saccharomyces* cells according to Jumiayati et al. (2013) cells of the yeast genus *Saccharomyces* are usually not ogival in shape, producing round or oval ascospores covered with asci which do not break easily and do not produce ballistospores [27]. It can be seen from the microscopic morphology of the yeast isolate that there are pseudohyphae and ascospores, so it is strongly suspected that this yeast isolate belongs to the genus *Saccharomyces*.

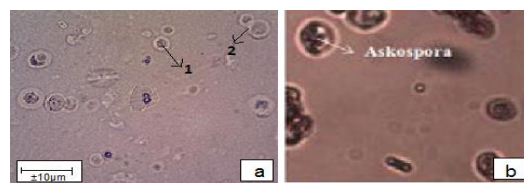


Fig. 7. a. Microscopic appearance of the genus *Saccharomyces* (1. Askospora, 2. Multilateral budding); b. Microscopic appearance of the genus *Saccharomyces* according to the literature.

3.5 Code B colony

The results of microscopic morphological observations of yeast B isolate showed that this colony has the characteristics of an oval or oval shaped colony, reproduces vegetatively by multilateral budding, measures $\pm 3\mu\text{m}$ and has no pseudohyphae. These microscopic features have the same shape as the yeast of the genus *Candida* sp. (Figure 8), based on Indrayati et al. (2018) which is known that the genus *Candida* sp. has the characteristics of an oval-shaped colony with a diameter of approximately $5\mu\text{m}$, reproduces by forming budding, besides that there are also found in the form of mycelium with pseudohyphae and sometimes found in the form of septate mycelium [25]. In addition, research conducted by Dewanto (2017) the genus *Candida* sp. showed elongated oval-shaped cells, measuring 2.73–2.93 μm , having 1 nucleus in the middle, forming short chains during division, and cells in small groups [28]. Widiastutik and Alami (2014) also conducted research by identifying the morphology of several yeasts, one of which was the genus *Candida* sp. with multilateral budding, do not form ascospores, arthrospores, teliospores, and ballistospores, but chlamydospores may form in some species [26]. The absence of capsules and pseudohyphae strengthens the notion that this isolate belongs to the genus *Candida* sp.



Fig. 8. Microscopic appearance of the genus *Candida* (1. Oval-shaped cells, 2. No ascospores, 3. Pseudohyphae, 4.

Multilateral budding); b. Microscopic appearance of the genus *Saccharomyces* according to the literature.

4 Conclusion

Based on the results of the study found as many as 5 isolates of fungi and yeast were isolated from the contaminants contained in simplicia cabe jamu in Madura. Morphological identification showed that the isolates of fungi and yeast found belonged to 3 genera of fungi namely *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. Also 2 genus yeast, namely *Saccharomyces sp.* and *Candida sp.* The results of this study provide an illustration that the samples of cabe jamu simplicia observed had poor sanitation.

The highest appreciation is expressed to LPPM of Trunojoyo University Madura (UTM) that have fully funded this research and publication (DIPA-023.17.2.677535/2022).

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