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Effect of Drying Pretreatment Methods on Amla (*Emblica officinalis*) Extracts Obtained Through Maceration Using Ethanol as Solvent

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Abstract. This study investigated the effects of drying methods on the yield, bioactive compounds, and antibacterial activity of amla extract. Fresh amla was oven-dried and sun-dried at different temperatures and ground into powder. Bioactive compounds were extracted from amla using maceration with ethanol as a solvent. The amla extract was used for the antibacterial susceptibility test using the agar disc diffusion method. The yield of amla extract increased with increasing drying temperature. The highest yield (53.47%) was obtained at a drying temperature of 70 °C. Fourier Transform Infrared (FTIR) analysis confirmed that sun-drying and oven-drying exhibited no qualitative effect on the bioactive compound in amla extract. FTIR analysis also indicated that amla extract contains bioactive compounds, validated by phytochemical analysis. The antibacterial activity of oven-dried samples at 40 °C produced the largest inhibition zone (24.57 mm) compared to sun-drying and oven-drying at other temperatures. It can be concluded that drying temperature, especially higher temperatures, had a significant impact on the antibacterial activity of amla as its active components degraded.

Keywords: Amla extract; Antibacterial activity; Bioactive compound; Drying methods

1. Introduction

Amla (*Emblica officinalis*) has long been recognized for its health benefits and has been used as a medicinal plant and in food preparation. It is currently distributed in India, Sri Lanka, South-East Asia, China, and Indonesia (Khan, Qais, and Ahmad, 2019) and is available in large quantities for food and pharmaceutical purposes. Amla has been extensively used as a medicinal plant to treat inflammation, as an antibacterial, and to treat cancer (Pareek and Kitinoja, 2011). A previous study has reported that amla has high pharmaceutical activity, sparking interest due to its potential as an antibacterial source (Jahan and Akter, 2015).

The potential of amla as a medicinal plant is mostly due to the bioactive compounds contained in the plant, which can treat various diseases in the human body. The bioactive compounds include flavonoids, tannins, alkaloids, saponins, and phenolic compounds (Arina and Harisun, 2019). Hussein, Mamman, and Mansur (2015) reported that the

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therapeutic effects of these medicinal plants are usually marked by the presence of secondary metabolites, which differ from one plant to another. Phytochemicals are secondary metabolites naturally present in plants (Guluma *et al.*, 2020). These phytochemical compounds act as natural antioxidants and can inhibit the formation of free radicals and lipid peroxidation (Ajak *et al.*, 2020). Phytochemical compounds such as flavonoids also have antimicrobial properties (Abdullah *et al.*, 2019). Bin Fazal and Ahmad (2024) recently reported on the potential use of amla extract as a capping and reducing agent in the synthesis of zinc-oxide nanoparticles, suggesting it as an alternative to surfactants.

Bioactive compounds, mainly phytochemical content from amla, are extremely important for many purposes. The phytochemical content of amla extract is heavily influenced by how the extract is produced. Maceration is an extraction method commonly used to extract bioactive compounds from plant materials through the use of solvents (solid–liquid extraction) (Safdar *et al.*, 2017). Ethanol is one of the most widely used solvents for the extraction of plant bioactive compounds, such as alkaloids and flavonoids (Pratiwi, Utami, and Arbianti, 2020). Ethanol is also considered a food-grade solvent (Dianursanti *et al.*, 2020).

The extraction of bioactive compounds from a plant begins with the drying of the plant. Plant drying aims to produce raw materials in simplicia, facilitating the extraction of phytochemicals with a high yield, and facilitating plant storage for future use (Amir *et al.*, 2021; Hasmita *et al.*, 2015). Additionally, drying significantly reduces volume, thereby saving on packaging, storage, and transportation costs (Sonkar *et al.*, 2020). Gudapaty *et al.* (2010) reported that drying affects the quality attributes of amla. The drying pretreatment method can affect the input energy, increase the extraction yield, and increase the quality of the extracted bioactive compounds. Thus, drying methods must be evaluated to determine their effect on extracted bioactive compounds.

For continuous production of amla extract, drying techniques must be determined for recommendation to producers. Raaf *et al.* (2022) reported the impact of temperature and drying methods on amla's drying kinetics and microstructure. However, little information has been reported regarding the effect of the drying method on bioactive compounds. Thus, this research evaluates the effects of oven-drying (at different temperatures) and sun-drying on the phytochemical content and antibacterial activity of amla.

2. Methods

2.1. Materials

The study used amla collected near Blang Bintang, Aceh Besar. The solvent used for the extraction process was ethanol 96% (Merck, Germany). The antibacterial assay used nutrient agar (Oxoid, UK) and amoxicillin (Mersi, Indonesia). The bacterial species used were *Staphylococcus aureus* ATCC 25923, obtained from stock cultures of Fundament Lab Sains, Aceh Besar.

2.2. Drying Methods

Fresh amla was washed with clean water and thinly sliced and dried. Cutting was performed to ensure that the amla samples dried evenly. The amla was sun-dried (SD) in air temperatures of 37–45 ℃. For oven-drying (OD), amla was placed on a tray and dried at four different temperatures (40 ℃, 50 ℃, 60 ℃, and 70 ℃). All samples were ground into a fine powder using an electric blender and screened through a mesh sieve to obtain a uniform size.

2.3. Extraction Procedure

The extraction method used in this study was maceration at room temperature. An illustration of the amla maceration process is presented in Figure 1. Dried amla (30 g) from each drying method was soaked in 150 mL ethanol 96% for 72 h. Maceration at room temperature (28 °C) was conducted in an Erlenmeyer flask covered with aluminum foil. The resulting mixture was filtered with Whatman no. 1 filter paper and concentrated using a rotary evaporator in a vacuum at 40 °C. The yield of each extract was determined by Equation (1).

$$\text{Yield (\%)} = \frac{\text{weight of amla extract}}{\text{weight of dried amla powder}} \times 100 \quad (1)$$

2.4. Analysis Method

Proximate analysis of amla powder was conducted to analyze parameters such as fat content, carbohydrates, protein, moisture, and ash using the standard method as per Indonesian National Standard (SNI) 01-2891-1992 procedures.

The preliminary phytochemical evaluation of each extract was qualitatively tested to determine the presence of flavonoids, alkaloids, tannins, saponin, quinone, steroids, and triterpenoids. The tests were performed according to the methods reported by Guluma *et al.* (2020).

A Fourier transform infrared spectrophotometer (FTIR Shimadzu Prestige 6400) was used to identify the functional group of amla extract. The spectra were observed in the wavenumber region of 4000–400 cm^{-1}. A Carl Zeiss-Bruker EVO MA10 scanning electron microscope (SEM) was used to observe the morphological changes of the dried amla powder after extraction. The analysis was presented using a 1000× magnification.

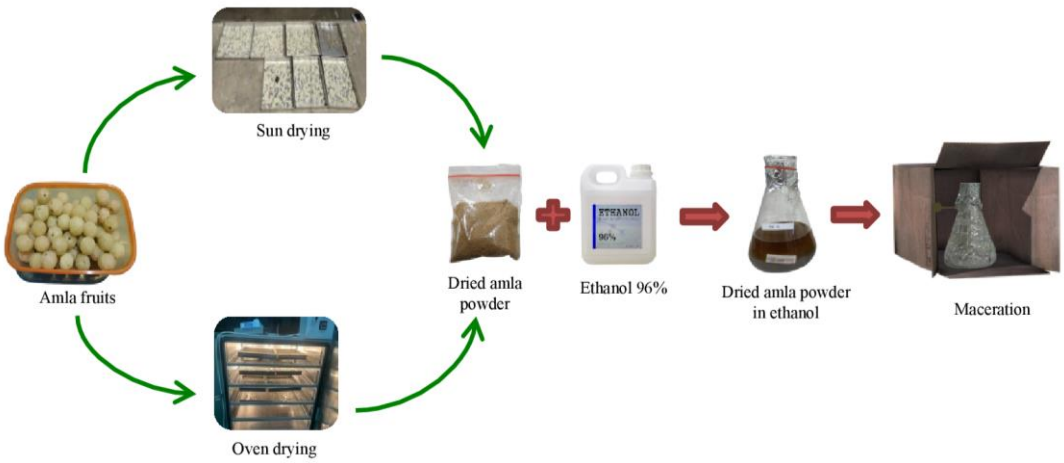

Figure 1 Illustration of the amla extraction process using maceration

2.5. Antibacterial Assay

The antibacterial activity of the amla extract was tested using the Kirby–Bauer agar disk diffusion method, as reported by Zullkiflee *et al.* (2022). *S. aureus* (ATCC 25923) was used in this study. The nutrient agar (NA) was used as the media. The NA was compacted in a Petri dish, and the bacterial culture was inoculated and diluted with 0.5 McFarland standard. The amla extract-soaked paper disk was drained and impregnated on the agar plate medium surface. The plates were incubated for 24 h at 37 °C. The clear zone around the paper disk was measured to determine the antibacterial activity. All results were compared to distilled water as a negative control and the standard antibiotic (amoxicillin) as a positive control.

3. Results and Discussion

3.1. Proximate Analysis of Dried Amla Powder

The proximate composition of dried amla powder is presented in Table 1. The proximate analysis results indicate that the carbohydrate and ash values are similar to those reported by Mishra and Mahanta (2014) for amla fruit powder; the fat and protein content was lower. This is a result of fruit from different geographical locations, which affects the nutritional content of the fruit, including fat and protein, as reported by Okeke *et al.* (2021). The fat content of amla ranged from 0.36% to 0.48% (Parveen and Khatkar, 2015). The protein content in amla is used by the body for growth and maintenance. Protein also plays a role in the formation of blood cells and antibodies that protect the body from disease and infection (Hermann, 2019). The obtained moisture content was high (10.08%). A less than 10% moisture content must be maintained to prevent microbial growth in dry food products (Zambrano *et al.*, 2019). Thus, proper pretreatment is required to limit the amount of moisture in dried amla powder without reducing its nutritional value.

Table 1 Proximate analysis of dried amla powder

Parameter	Content (%)
Moisture	10.08
Ash	3.57
Fat	0.15
Protein	4.43
Carbohydrates	81.77

3.2. Effect of Drying Methods on Amla Extract Yield

The effect of the drying method on the yield of amla extract is presented in Figure 2. Oven-drying at 50–70 °C produced a higher amla extract yield than sun-drying. The amla extract yield was slightly higher with sun-drying than with oven-drying at 40 °C as the drying temperature was 40–45 °C. This indicates that drying methods have a linear effect on the increase in amla extract yield. These results are consistent with those reported by Justine *et al.* (2019).

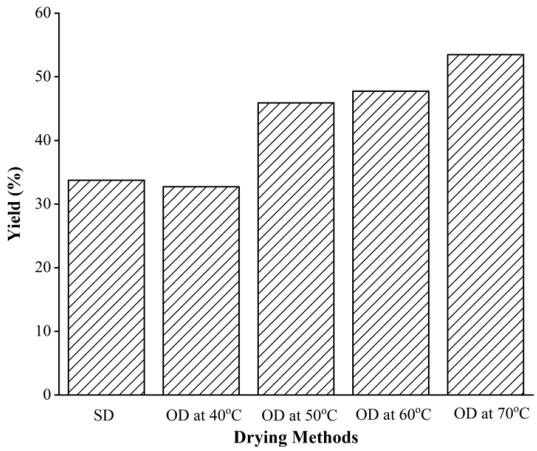

Figure 2 Effects of sun-drying (SD) and oven-drying (OD) on amla extract yield

The amla extract yield increased linearly with increasing drying temperature, related to the moisture content in the amla. Higher drying temperatures produced a lower moisture content in the amla (Raaf *et al.*, 2022). Thermal drying causes cell wall damage, facilitating the release of phytochemical compounds and increasing amla extract yield (Justine *et al.*, 2019). Cell wall damage caused by thermal drying is shown in Figure 3**.** Higher drying temperatures can increase cell wall damage (Figure 3B). Raaf *et al.* (2021) reported that the

amount of water in the material at high temperatures was smaller, resulting in the stretching of the plant cell wall structure, which facilitated the rupture of the cell wall into small particles.

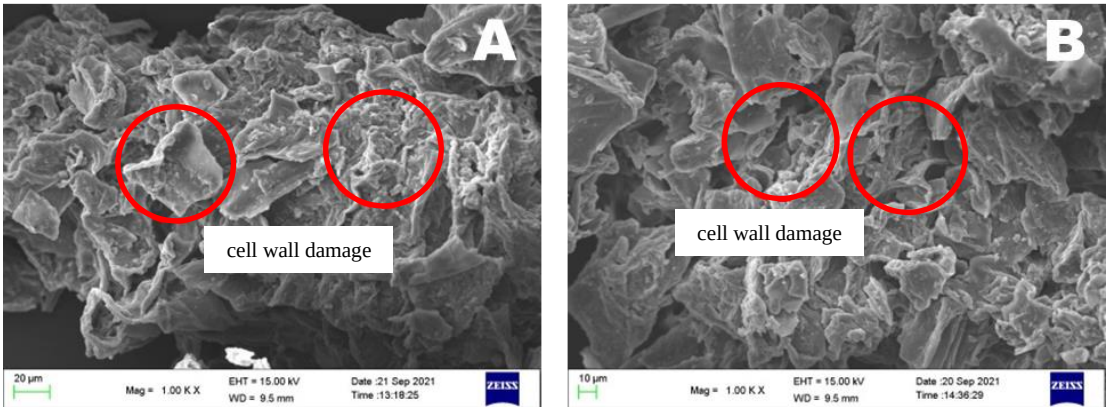

Figure 3 Effect of drying temperature on the cell wall damage after extraction: A: 50 °C; B: 70 °C

The effect of the extraction process on cell wall damage is presented in Figure 4. Figure 4A shows the cell wall damage caused by thermal drying pretreatment. Figure 4B shows the cell wall damage caused by ethanol penetration during the extraction process. The pretreatment drying damaged the amla cell wall, facilitating ethanol to more easily extract phytochemical compounds from amla cells (Dianursanti *et al.*, 2020). This is also characterized by greater cell wall damage after extraction.

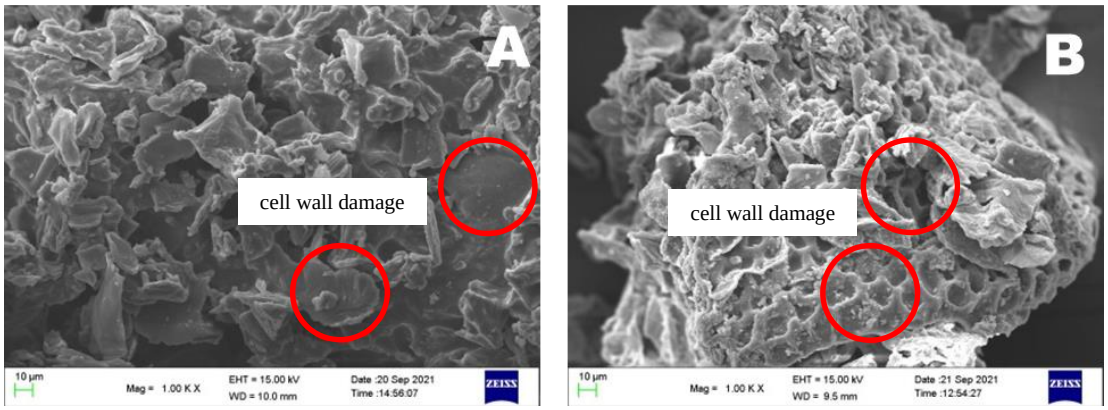

Figure 4 Effect of extraction on cell wall damage: A: before extraction (after oven-drying at 60 °C); B: after extraction

The effect of the drying method on cell wall damage after extraction is presented in Figure 5. Sun-drying (Figure 5A) and oven-drying at 40 °C (Figure 5B) have similar thermal temperatures, with a difference of 5 °C. Cell wall damage caused by thermal drying is difficult to determine. The amla extract yield was 1% greater with sun-drying than with oven-drying at 40 °C (Figure 2). Cell wall damage after extraction appeared to be greater with oven-drying at 40 °C than sun-drying. Oven-drying has a closed drying system; sun-drying has an open drying system. There is less heat loss in oven-drying than sun-drying (Babu *et al.* 2018). Thus, oven-drying at 40 °C (Figure 5B) observed greater cell wall damage than sun-drying (Figure 5A).

3.3. Effect of Drying Method on Phytochemical Analysis of Amla Extract

Amla extract has a high polyphenols content (Kumari and Khatkar, 2016). Polyphenols are natural phytochemical compounds in plants. Chemically, polyphenols have one or more

phenol groups. The phenol group comprises one or more hydroxyl groups (-OH) bonded to an aromatic ring (Aneklaphakij *et al.*, 2021). Polyphenols are also known as phenolic compounds. Polyphenols can inhibit bacterial growth (Aneklaphakij *et al.*, 2021) because they contain flavonoids, saponins, and tannins as bioactive compounds (Arina and Harisun, 2019). Thus, amla extract is promising as an antibacterial agent (Variya, Bakrania, and Patel, 2016). The bioactive compounds in amla extract must be identified. This study used FTIR analysis to identify the bioactive compounds in amla extract based on their functional groups. The FTIR spectrum of amla extract is presented in Figure 6.

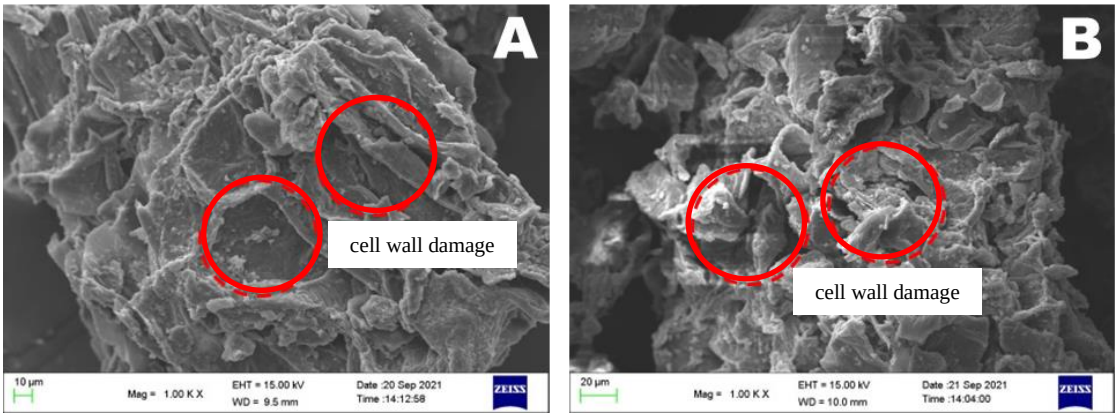

Figure 5 Effect of drying method on cell wall damage after extraction: A: sun-drying; B: oven-drying at 40 °C

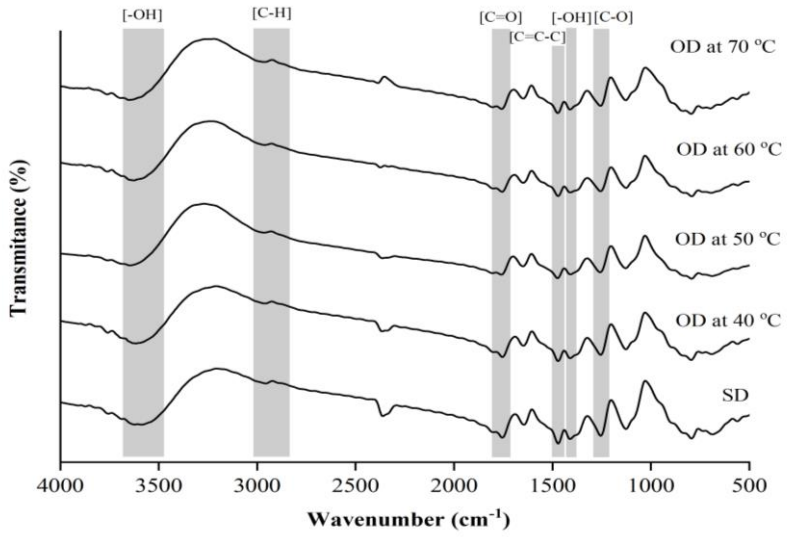

Figure 6 FTIR spectrum of amla extract (sun-drying (SD) and oven drying (OD) at 40–70°C)

The FTIR spectra of amla extract exhibited several main peaks. The peak at 3652–3588 cm^{-1} indicates the presence of –OH (stretching) vibration of the phenol group (Nandiyanto, Oktiani, and Ragadhita, 2019). The C-H vibration was found at 2964–2957 cm^{-1}. It exhibits phenolic aromatic compounds (Öztürk *et al.*, 2019). The peak at 1759–1754 cm^{-1} shows the ester group's C=O (stretching) vibration (Nandiyanto, Oktiani, and Ragadhita, 2019). Peaks at 1473–1470 cm^{-1}, 1410–1409 cm^{-1}, and 1257–1254 cm^{-1} indicate C=C-C (stretching) vibration of the aromatic ring, -OH (bending) of the phenol group, and C-O (stretching) of the phenol aromatic ring, respectively (Nandiyanto, Oktiani, and Ragadhita, 2019). Overall, the FTIR spectra exhibited good consistency with the results reported by Firdous, Ringø, and Elumalai (2020). Polyphenol compounds are indicated by the functional groups of –OH, C=O, and C-O (Raaf *et al.*, 2021). This ester group indicates the presence of flavonoids (Noh,

Azmin, and Amid, 2017), saponins (Almutairi and Ali, 2015), and tannins (Grasel, Ferrão, and Wolf, 2016).

The results are also supported and confirmed by identification using the reagents presented in Table 2. Flavonoids, saponins, and tannins were qualitatively identified through FTIR and phytochemically by reagents in amla extract under each drying condition. The results indicated that sun-drying and oven-drying had no qualitative effect on the phytochemical compounds in amla extract.

Table 2 Phytochemical analysis of amla extract

Constituent	Drying Method				
	SD	OD 40 °C	OD 50 °C	OD 60 °C	OD 70 °C
Flavonoid	+	+	+	+	+
Alkaloid	-	-	-	-	-
Tannin	+	+	+	+	+
Saponin	+	+	+	+	+
Quinone	-	-	-	-	-
Steroid	-	-	-	-	-
Triterpenoid	-	-	-	-	-

+: Available
-: Unavailable

3.4. Antibacterial Activity

In this study, amla extract was used as an antibacterial agent. The bioactive compounds in amla extract can form complexes with bacterial cell walls, which disrupt bacterial cell membranes. Kumari and Khatkar (2016) also observed the antimicrobial activity of amla extract on several bacterial species, including *Staphylococcus aureus (S. aureus)*. The antibacterial activity of amla extract on *S. aureus* with different drying methods is shown in Figure 7 and Table 3.

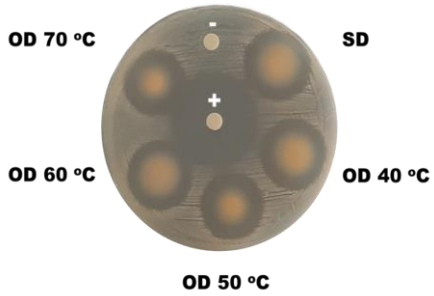

Figure 7 Inhibition of *S. aureus* growth by amla extract using sun-drying (SD) and oven drying (OD) at 40–70°C

The antibacterial activity of oven-dried samples (40 °C) was shown to be greater than that of other oven-dried (50 °C, 60 °C, 70 °C) and sun-dried samples. Compared with the other treatments, drying at 40 °C exhibited the greatest inhibitory activity, with an inhibition zone of 24.57 mm against gram-positive bacteria *S. aureus*. The results indicate that amla extract has reasonable antibacterial activity against the test microorganisms regardless of the drying method. The antibacterial activity of amla extract on *S. aureus* was greater than that previously reported by Kumari and Khatkar (2016).

The inhibition zones indicated the test organisms' susceptibility to amla extract and decreased as the amla drying temperature increased. This is reasonable; Hussein, Mamman, and Mansur (2015) reported that *M. oleifera* leaf extract was more effective at low temperatures. The presence of tannins (Khan, Qais, and Ahmad, 2019) and flavonoids (Abdullah *et al.*, 2019) as bioactive components is thought to be responsible for the antibacterial activity of amla extract.

Table 3 Effect of drying method on the antibacterial activity of amla extract

Drying method	Zone of Inhibition (mm)
Sun-drying	24.21 ± 1.01
Oven-drying, 40 °C	24.57 ± 1.10
Oven-drying, 50 °C	23.28 ± 0.86
Oven-drying, 60 °C	23.89 ± 0.93
Oven-drying, 70 °C	23.14 ± 0.92
Amoxicillin (positive control)	31.82 ± 1.27
Aquadest (negative control)	0
Cabinet drying (Kumari and Khatkar, 2016)	20.01 ± 0.12

4. Conclusions

The drying method significantly affected the yield of amla extract, which was slightly greater with sun-drying than with oven-drying at 40 °C and increased with increasing drying temperature. Sun-drying and oven-drying methods caused cell wall damage in dried amla powder before extraction. The extraction process also causes cell wall damage due to solvent activity, which releases phytochemical compounds from dried amla powder. FTIR and phytochemical analysis indicated that amla extract contains flavonoids, tannins, and saponins. The presence of an inhibition zone indicated that amla extract demonstrated antibacterial activity. The antibacterial activity of amla extract on *S. aureus* decreased with increasing amla drying temperature.

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