FOODSCITECH
Food Science and Technology Journal

Phytochemical Tests and Antioxidant Activity Tests From Ethanol Extract Of Fig Leaves (*Ficus carica* Linn.) Green Yordan

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ABSTRACT

The fig plant (Ficus carica L.) is one of the Moraceae family, which usually grows in countries with tropical and subtropical regions whose leaves have the potential as traditional medicine and also as an antioxidant. The purpose of this study was to identify the phytochemical content and antioxidant compounds from the ethanol extract of fig leaves of the Green Jordan variety. Active compounds were extracted by the maceration method using ethanol solvent with concentrations of 50%, 60%, 70%, and 96%. Phytochemical tests include testing for alkaloids, flavonoids, saponins, and tannins. The antioxidant activity test was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The antioxidant activity test was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The results of the phytochemical test showed that the secondary metabolites contained in the ethanol extract with various concentrations of Fig leaves include alkaloids, flavonoids, saponins, and tannins In addition, Fig leaves extract with 96% ethanol solvent has a very strong antioxidant potential of 96.0%.

Keywords: Antioxidant, Fig Leaves (*Ficus carica* L.), DPPH, Phytochemical test

INTRODUCTION

Medicinal plants and plants are a source of medicinal ingredients that the Indonesian people have traditionally used for generations to treat diseases. The use of natural ingredients as traditional medicine in Indonesia is increasing because it is considered to have lower side effects than synthetic drugs from chemicals, and the price is also more affordable. (Dewi, Hakim, & Savalas, 2018). Ammar et al. (2015) carried out a study that identified 116 phenolic compounds in the leaves, fruit, skins, and pulps of two fig varieties (green and black) sourced from Tunisia. The research indicated that the leaves and skin of the black varieties possessed a diverse qualitative polyphenolic profile, with rutin being the predominant element in fruits, skins, and leaves, whereas prenylhydroxygenistein was the key component in pulp.

Analysis showed the presence of nine types of anthocyanins, of which cyanidin 3-O-rutinoside and cyanidin 3,5-diglucoside were the dominant compounds. Two of these were found in green varieties. Rutinoside has been recognized as an important compound in multiple fig varieties from diverse geographical areas. Anthocyanins, which are naturally occurring pigments that give many fruits, flowers, and vegetables their red, purple, or blue hues, include cyanidin 3-O-rutinoside. This chemical is structurally composed of the aglycone cyanidin attached to the sugar rutinose by a glycosidic link. Strong antioxidant qualities, the capacity to stabilize free radicals, and the potential to offer anti-inflammatory and degenerative disease prevention are all attributes of cyanidin 3-O-rutinoside. One kind of anthocyanin is cyanidin 3,5-diglucoside, commonly referred to as cyanin. Natural pigments called anthocyanins give a variety of fruits, flowers, and vegetables their red, purple, or blue hues. A cyanidin aglycone is structurally attached to two glucose molecules at positions three and five to form cyanidin 3,5-diglucoside.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.8656

Fig leaves (Ficus carica L.) have long been used in traditional medicine. The general populace frequently uses them to treat a range of illnesses, such as managing diabetes, reducing kidney stone symptoms, and serving as a natural laxative. Furthermore, fig leaves are well known for their ability to reduce congestion, encourage diuresis, and aid in ulcer healing. They are a useful tool in the field of natural medicines because of their acknowledged antitumor and anticancer qualities. (Sirisha, Sreenivasulu, Sangeeta, & Madhusudhana Chetty, 2010). It is also used to treat inflammation, paralysis, liver and spleen disease, chest pain, headaches, leprosy, nosebleeds, and hair growth. (Verma, Gupta, Rajinder, & Gupta, 2015). An ethnobotanical survey of medicinal plants conducted by Barkaoui et al. (2017) indicated that the fruits and leaves of figs are used by practitioners to treat diabetes complications in Morocco

Based on several studies that have been carried out on the leaves of the fig (*Ficus carica* L.) plant, the antioxidant activity test using the DPPH method of extract of fig (*Ficus carica* L.)

Depending on the extraction solvent, the Brown Tukey cultivar has antioxidant activity at different quantities. 78.13 g/mL, 492.80 g/mL, 16 g/mL, 40 g/mL, and 129.18 g/mL were the IC_{50} values for the methanol extract, n-hexane, ethyl acetate, n-butanol, and water, respectively. With the ethyl acetate fraction exhibiting the highest antioxidant potency, these figures show the various degrees of antioxidant activity found in the various extracts.(Prayoga & Rahmawati, 2019). According to (Nafi'ah & Nurulhuda, 2020), fig leaves extract with methanol solvent has antioxidant activity with an IC_{50} value of 3.3005 g/mL, Fig leaves extract with water solvent has an antioxidant activity of 3.6976 g/mL, and fig leaves extract with methanol: water solvent has antioxidant activity 13.6140 g/mL. Based on the results of previous studies, the fig used is brown Tukey and uses methanol solvent, and the use of ethanol solvents is rather rare, so researchers want to use ethanol solvents. According to (Azizah & Salamah, 2013), Ethanol solvent can also extract more chemical compounds than water and methanol. So that researchers are interested in raising the topic of this research which aims to determine the phytochemical content contained in the ethanol extract of Tin (*Ficus carica* L) leaves of the Green Jordan variety and to choose the antioxidant activity in vitro so that it can be used as a scientific basis for proving empirical treatment that has been carried out so far. This research is expected to obtain compounds that are efficacious as antioxidants and so that it becomes the first step for further research that can be developed in pharmaceutical science. Thus, it can increase the added value of using tin fruit (*Ficus carica* L.) Green Jordan.

METHODS

Sample preparation

Fig leaves (*Ficus carica* L) of the Green Yordan variety were obtained from Gavin's Fig, in the Grobogan area of Central Java. The fig leaves are sorted, washed clean, and dried for five days. The dried fig leaves are mashed using a blender until they become fig leaves powder.

Reagents

Chemicals used include ethanol (50%, 60%, 70%, 96%), distilled water, DPPH (Sigma Aldrich), Wagner's reagent, 1% $FeCl_3$, 2M HCl, NaCl, magnesium powder, and concentrated HCl.

Extraction process

The dried fig leaves are mashed using a blender until they become fig leaves powder. The fig powder used in maceration is 50 g with 300 mL of ethanol solvent with occasional stirring, The maceration process with various concentrations of 50%, 60%, 70%, and 96% ethanol solvent at room temperature for 24 hours. After the maceration process, filtering was carried out with filter

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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paper to separate the filtrate and residue. The residue from the maceration was macerated again with a suitable solvent for 24 hours. Simultaneously, the filtrate was collected first. The results of the remaceration are also separated. The filtrate from the remaceration is combined with the first filtrate. The filtrate was then concentrated using a rotary evaporator at a certain temperature and pressure until a thick extract was obtained. After that, several phytochemical tests were carried out, namely alkaloids, flavonoids, saponins and tannins, and antioxidant activity tests.

a. Alkaloid Test

Testing for alkaloids using the Wagner method, namely taking 2 mL of sample and adding 2M HCl, then heated in a water bath while stirring, then cooled to room temperature. Add NaCl powder, mix, and filter, then add the filtrate with 2M HCl to a certain volume. The filtrate was divided into 2 test tubes, the first tube was added with Wagner reagent, and the other line was used as a blank. The first tube was observed to form a precipitate and compared with the blank. The positive substance contains alkaloids if a deposit is created (Dey et al., 2020).

b. Flavonoid Test

As much as 2 mL of fig leaves ethanol extract was taken, adding 0.5 grams of magnesium powder and ten drops of concentrated hydrochloric acid. The presence of flavonoids is indicated by the formation of a reddish black, yellow, or orange color (Goud & Prasad, 2020)

c. Saponin Test

The 2 mL sample was added with 5 mL of distilled water and then shaken so there was foam. If the foam persists for 30 seconds, then identification indicates the presence of saponins(Jaramillo-Carmona et al., 2017).

d. Tannin Test

The thick extract was added with 2 mL of distilled water, boiled for 5 minutes, and added with five drops of 1% $FeCl_3$. Observe the color changes that occur. The formation of dark blue or blackish green color indicates the presence of tannins (Dykes, 2019).

Antioxidant Activity Test

A scientific technique for assessing a substance's capacity to combat free radicals—unstable chemicals that might harm cells through oxidative stress—is antioxidant testing. Antioxidant testing is primarily used to determine and quantify the antioxidant capacity of manufactured or natural substances, including chemicals, medicines, and plant extracts. The ability of a material to stop or reduce the oxidative damage that free radicals and reactive oxygen species (ROS) cause to biological systems is known as antioxidant activity. Understanding the preventive capacity of natural or synthesized substances and their function in averting aging, chronic diseases, and other health problems associated with oxidative damage.

The thick extract of Fig leaves was tested for its antioxidant activity by the DPPH method. The antioxidant activity test or RSA (Free Radical Scavenging Activity) was carried out based on Prayoga & Rahmawati (2019). An antioxidant test was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). Each 100µl filtrate was put into a small test tube plus 2.9 mL of DPPH (0.004% concentration in methanol). After 120 minutes of incubation at room temperature and without light, the absorbance of the solution was measured at the maximum absorption wavelength of DPPH (515 nm).

Data Analysis

The research data were analyzed using an experimental design, namely RAL (Completely Randomized Design), with one factor, solvent concentration, repeated three times so that 12

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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experimental units were obtained. The analysis results were continued with the Honest Significant Difference (BNJ) test at the 5% level.

RESULT AND DISCUSSION

Phytochemical Test of Green Jordan Varieties of Fig leaves Extract

In order to determine the active substances found in plants and gain an understanding of their bioactive potential, phytochemical studies were carried out. Because of its polarity, which makes it extremely successful in dissolving and extracting phenolic chemicals from fig leaves (Tin), ethanol was chosen as the extraction solvent in this work. The polarity of ethanol makes it easier to extract hydroxyl-rich substances like flavonoids and phenolics, which are important components of the plant's antioxidant and medicinal qualities (Hidayah, 2013).

It is commonly known that ethanol has better extraction properties than other solvents like methanol and water. Its ability to dissolve a wider variety of chemical compounds, including both polar and slightly non-polar molecules, is due to its intermediate polarity. Ethanol is therefore a perfect solvent for phytochemical research since it can efficiently extract a wide range of bioactive substances, including phenolics, flavonoids, and alkaloids. Ethanol is also widely used in research and industry since it is thought to be safer and more ecologically friendly than methanol (Azizah & Salamah, 2013).

The test is carried out by taking a small sample of the maceration extract and then adding the reagent according to the compound to be determined. Based on the results of phytochemical tests, it was found that fig leaves extract contains flavonoids, alkaloids, tannins, and saponins (see table 1).

Table 1. Phytochemical Test Results of Fig Leaves Ethanol Extract

Phytochemical Test	Indicator	Ethanol Concentration (%)			
		50	60	70	96
Alkaloids	Brown precipitate is formed	(+)	(+)	(+)	(+)
Flavonoids	Color change to reddish-black	(+)	(+)	(+)	(+)
Saponins	Stable foam is formed	(+)	(+)	(+)	(+)
Tannins	Color change to black	(+)	(+)	(+)	(+)

The test for alkaloids of fig leaves extract of the Green Jordan variety using Ethanol with several concentrations (Table 1, Figure 1) showed that all samples showed positive results. This follows the statement Marliana, Suryanti, & Suyono (2005) that the Wagner test is said to be positive if a light brown to yellow precipitate is formed. The sediment is considered a potassium-alkaloid. While preparing Wagner's reagent, iodine reacts with I^- ions of potassium iodide to give brown I_3^- ions. In Wagner's experiment, metal ions K will form coordinate covalent bonds with nitrogen in the alkaloids to form a precipitated potassium-alkaloid complex. The presence of alkaloids in *Ficus carica* Linn. Green Yordan shows that the leaf powder has various pharmacological qualities, including antihypertensive, antiarrhythmic, anti-malaria, and anti-cancer activities (Adeniyi, et al. 2009), which is consistent with Saxena, et al. (2012).

FOODSCITECH, 7 No. 2 December 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.8656

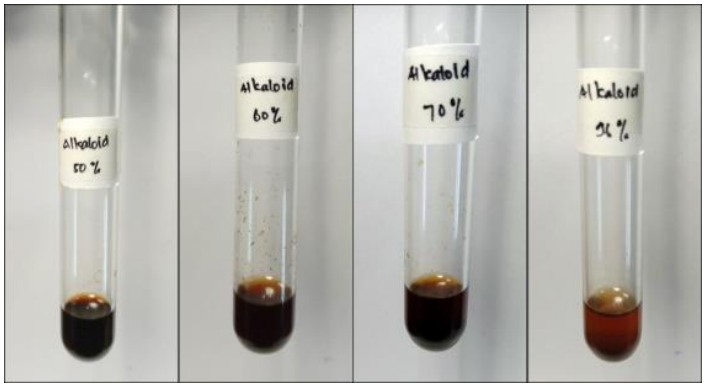

Figure 1. Alkaloid Test

In the flavonoid test results (Table 1, Figure 2), the sample gave a positive outcome: the color changed to reddish black. The development of a quinoidal structure is responsible for the red coloration seen in the flavonoid test. The carbonyl group at the C-4 position of flavonoids is reduced to create a hydroxyl group when they are treated with magnesium and hydrochloric acid. Together with the preexisting hydroxyl groups on the A ring, this freshly created hydroxyl group takes part in a number of proton transfers and tautomerizations. This absorption of visible light and the subsequent development of a red color are caused by the expanded conjugated system in the ensuing quinoidal structure.

Flavonoids are polar compounds because they have many hydroxyl groups. Therefore, flavonoids are usually soluble in polar solvents such as Ethanol. Ethanol functions as a reducing agent for flavonoids from its salt form. The addition of concentrated hydrochloric acid protonates flavonoids to form flavonoid salts. After adding magnesium powder, a positive result was indicated by a change in the color of the solution to red-black. The reddish-black color indicates flavonoids' presence on reduction with concentrated hydrochloric acid and magnesium.

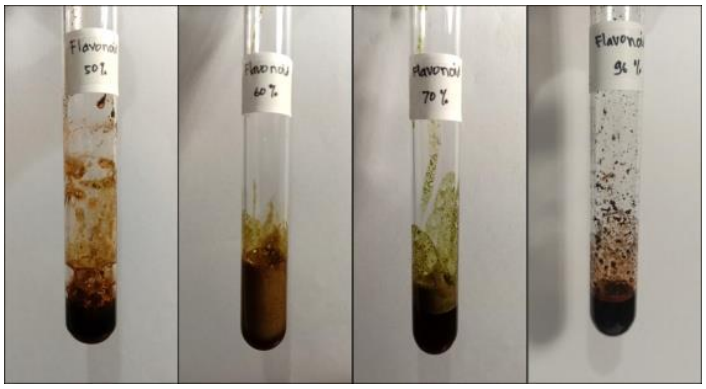

Figure 2. Flavonoid Test Results

The process of extracting and identifying saponin components from a sample is known as saponin testing with ethanol. Because it can dissolve saponins, which are amphipathic glycosides made up of a hydrophilic sugar moiety and a hydrophobic aglycone (sapogenin), ethanol is frequently used as a solvent. To obtain the saponin-rich extract, the sample is usually combined with ethanol, then agitated and filtered. After that, the extract can undergo quantitative examination using sophisticated methods like spectrophotometry or chromatography, or qualitative testing like the development of stable foam upon shaking.

Saponins are glycosides with a hydroxyl group in their molecule with the formula $C_{32}H_{18}O_7$. Saponins have soap-like properties, forming foam or foam when dissolved in water. Fig leaves extract with several treatments of ethanol concentration was said to be positive for saponins

FOODSCITECH, 7 No. 2 December 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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because the foam produced remained stable for 30 minutes. The test for saponin of fig leaves extract of the Green Jordan variety using Ethanol with several concentrations (Table 1, Figure 3 showed that all samples showed positive results. This is because saponins are soap-like compounds with hydrophilic and hydrophobic groups that can act as active surfaces in foam formation. Foaming can occur because saponins have two opposite properties in their structure, polar glycosides, and non-polar steroid ring. When shaken with water, only glycosides will bind water while steroids repel water. This is the principle of foam formation (Heliawati, 2018). Saponins are dietary supplements that also have antimicrobial activity. They are useful in modulating. Improves blood lipids, lowers cancer risk, boosts blood glucose response, and has antioxidant capabilities.

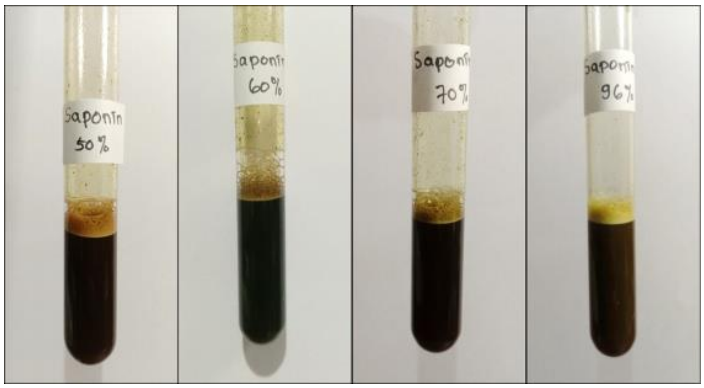

Figure 3. Saponin Test Results

Tannins are compounds containing hydroxyl groups (derived from benzene) that are soluble in water due to hydrogen bonds between the hydroxyl groups of tannins and water molecules. The heating process also impacts breaking the bonds in the tannins so that free tannin monomers are formed. Adding 1%, the $FeCl_3$ reagent changed the color from greenish-brown to greenish-black. The addition of 1% $FeCl_3$ was used to determine the content of phenol compounds. When $FeCl_3$ is added to a solution containing tannin, a complexation reaction between the Fe^{3+} ion and the phenolic hydroxyl group on the tannin molecule will occur. This reaction will result in a complex compound that is dark blue or dark green. A color change indicated the presence of a phenol group to dark green or dark blue after adding 1% $FeCl_3$. The test for tannin of fig leaves extract of the Green Jordan variety using Ethanol with several concentrations (Table 1, Figure 4) showed that all samples showed positive results A positive tannin test suggests the presence of phenolic compounds; one of them is tannins because tannins are polyphenolic compounds.

Plants that contain tannins have been effectively employed as astringents, as diuretics, to combat stomach and duodenal cancers (Saxena, et al. 2012) whereas flora that include flavonoids offer health advantages like antioxidants and anti-inflammatory agents impacts (Saxena, et al. 2013). The ability of tannins, a type of polyphenolic chemicals, to bind proteins and other macromolecules adds to their therapeutic qualities and makes them useful in both conventional and alternative medicine. Flavonoid-rich plants, on the other hand, provide an equally remarkable array of health advantages, serving as potent antioxidants that counteract dangerous free radicals and as anti-inflammatory agents that lessen chronic inflammation and the illnesses that are linked to it. These bioactive substances highlight the enormous potential of plant-based compounds to improve human health and fend against a variety of illnesses, underscoring the need of investigating natural

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.8656

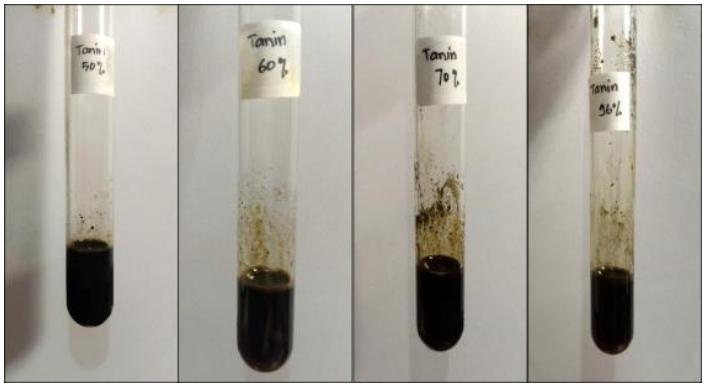

Figure 4. Tannin Test Results

4.2. Antioxidant Test Method DPPH

The analysis of diversity in the antioxidant activity parameters shows that the higher the concentration of ethanol solvent, Antioxidant activity is characterized by the IC_{50} value,which is the concentration of test solution required to inhibit 50% of DPPH free radicals. Activity antioxidant activity of a compound can be classified based on the IC_{50} value obtained. If the IC_{50} value value of an extract <50 ppm then the antioxidant activity is category is very strong, the IC_{50} value is between 50-100 ppm means the antioxidant activity is strong category, IC_{50} value is between 100-150 ppm means antioxidant activity medium category, IC_{50} value is between 150-200 ppm means the activity antioxidant activity weak category, while if IC_{50} value > 200 ppm then the antioxidant activity categorized as very weak the more likely the antioxidant activity to increase, as shown in Table 2. below.

Table 2. Antioxidant Activity

Ethanol Concentration (%)	Antioxidant Activity (%)
50	81.4
60	95.5
70	95.5
96	96.0

According the result in the Table 2, the higher the concentration of ethanol solvent, the higher the antioxidant activity. The results of antioxidant activity testing of fig leaves ethanol extract using concentrations of 50, 60, 70, and 96 are shown in Table 2. Table 2 shows that the higher the concentration of the extract, the smaller the absorbance value and the higher the % antioxidant activity value. This is because the higher the concentration of the extract, the higher the concentration of compounds that have antioxidant properties so that the higher the inhibition of DPPH which causes the absorbance value to be lower because the remaining DPPH is smaller. In 96% ethanol extract obtained antioxidant activity of 96.0% which means fig leaves extract has a strong antioxidant activity category. good antioxidant substances which can act as antioxidant substances by preventing the negative effects of free radicals. This substance works as an antioxidant by donating its electrons.The value of antioxidant activity will increase depending on the increase in the total content of phenolic compounds and flavonoids present in the extract. Total phenols and flavonoids from fig leaves extract were positively correlated with antioxidant activity. (Dhianawaty & Ruslin, 2015).

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Reports indicated that DPPH is one of the substances that contained a proton reactive species exhibiting a distinct absorption, that reduces considerably upon exposure to extreme proton interceptors. The level of DPPH in the *Ficus carica* Linn. suggests that they can serve as a useful alternative to inorganic based antioxidants (Sirajo. 2018).

Phenolic compounds and flavonoids from fig leaves can be used as a source of antioxidants. Phenolic compounds, including flavonoids, can act as antioxidants because they contain a hydroxyl group bound to the carbon of the aromatic ring so that it can capture free radicals. (Tawfeek et al., 2021). Phenolic compounds can react with free radicals because of their ability to donate electrons (reducing), produce more stable products, and inhibit free radical chain reactions. (Plaza, De Torres, Lücking, Vizcaya, & Medina, 2014). The results showed that fig leaves extraction using 96% ethanol had the highest antioxidant activity of 95.68%.

CONCLUSION

Based on the results of the research and subsequent discussion, it was determined that all extracts of fig leaves from the Green Jordan variety, prepared using ethanol concentrations of 50%, 60%, 70%, and 96%, contain significant bioactive compounds, including alkaloids, flavonoids, saponins, and tannins. The fig leaf extracts have significant antioxidant potential, with the extract prepared using 96% ethanol achieving an antioxidant activity of 96%, designated as strong. This enhanced activity is attributed to the higher concentration of phenolic compounds and flavonoids, which are known to donate electrons, stabilize free radicals, and inhibit chain reactions of oxidation. The analysis of antioxidant activity parameters clearly shows a relationship between ethanol solvent concentration and antioxidant activity, with higher ethanol concentrations yielding stronger antioxidant activity, as indicated by the IC_{50} values.

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