

Antioxidant Activity and Phytochemical Screening of *Rhizophora mucronata* Mangrove Leaf Extract from Mangrove Botanical Garden, Surabaya City, East Java

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ABSTRACT *Rhizophora mucronata* is a type of mangrove plant that acts as a natural antioxidant which is used as a food ingredient and traditional medicine. The aim of this research was to determine the profile of secondary metabolites, antioxidant activity and total phenols of *R. mucronata* leaf extract. *R. mucronata* leaves were obtained from Gunung Anyar District, Surabaya City and then extracted using three solvents with different polarities, namely n-hexane, ethyl acetate, and ethanol. Mangrove leaf extract (*Rhizophora mucronata*) was followed by phytochemical testing using color testing, antioxidant activity testing using the DPPH method, and determining phenol content using the Folin-Ciocalteu method. Phytochemical tests show that all *R. mucronata* mangrove leaf extracts contain secondary metabolites such as flavonoids, saponins, steroids, triterpenoids and tannins with total phenols ranging from 0.04%-6.7%. The antioxidant activity of n-hexane, ethyl acetate, and ethanol extracts were 4.9 mg ± 0.82%, 1.7 mg ± 0.17%, and 0.21 mg ± 1.9%, respectively.

Keywords: Antioxidant; phenol; phytochemicals; *R. mucronata*

INTRODUCTION

Indonesia is an archipelagic country rich in natural resources. One of the natural wealth that can be utilized its natural potential is mangrove plants or commonly known as mangrove plants (Lasabuda, 2013). Mangroves are widespread in tropical and subtropical regions, mostly found along saltwater tidal zones of the Indo-Pacific protected coastline (India to Australia) (Sachithanandam et al., 2019). According to Triyatno et al. (2019) mangroves are referred to as coastal plants, tidal plants and brackish plants because they grow well on coral beaches or thin sandy coral reef land, or on beaches that have alluvial soil types. Estimated mangrove area in Indonesia is ±3 million hectares (Rahadian et al., 2019). Around 202 types of mangrove species in Indonesia have been identified and thrive because the tropical climate is an ideal place for mangrove plant growth (Purwaningsih et al., 2013). One type of mangrove that is abundant in tropical rainforests in Indonesia is *Rhizophora mucronata* (Lesdiana & Usman, 2021).

Rhizophora mucronata is a species of mangrove plant belonging to the Rhizophoraceae and has a large distribution in the Indo-Pacific region (Abdi et al., 2019). *Rhizophora mucronata* Or it can be called tinjang or tanjang has elliptical leaves that expand to an elongated round shape and have a tapered tip. The petioles are green, and the surface of the leaves is textured like bark. *R. mucronata* fruits have a brownish-green color, are oval or egg-like, and feel rough at the base. The roots have a root system of supporting roots (stilt-roots) (Angio et al., 2022). Morphologically, *R. mucronata* can grow up to a height of 3-4 meters and can produce cigar-shaped fruit (propagul) (Sadeer et al., 2019). Recent studies have shown that this plant extract acts as an antioxidant,

anti-diabetic, antimicrobial (anti-viral, anti-fungal, anti-bacterial), anti-cancer, anti-inflammatory, and analgesic (Setyawan et al., 2022).

Research related to the content of compounds in mangroves *Rhizophora mucronata* has been widely conducted. *Rhizophora mucronata* mangrove leaves contain 2-(2ethoxy ethanol, kau-16-ene and benzophenon, phenolic compounds flavonoid group, phenolic acids, tannins dihydroflavonol, caffeic acid, vanillic acid, p-hydroxy benzoate acid, tannins, alkaloids, coumarins, flavonoids, phenols and polyphenols, quinones, resins, saponins, phytosterols, xanthoprotins, pigments (chlorophyll, carotenoids) and sugars (Ridlo et al., 2017). Secondary metabolite compounds contained in *R. mucronata* mangrove leaves consist of phenols, flavonoids, tannins, saponins, terpenoids which are a response to a growing environment that is not ideal (Akasia et al., 2021). According to Mile et al. (2021), *R. mucronata* macerated with ethanol solvent showed an average total flavonoid content of 19.5095 mg/ml while phytochemical screening results were identified as containing alkaloids, flavonoids, tannins, saponins, and phenolic compounds.

Rhizophoraceae extract has high potential as a source of antioxidants that benefit human health by fighting free radicals (Indriaty et al., 2023). Antioxidants can play a role in overcoming degenerative diseases. This is evidenced by the increase in antioxidants such as the enzyme catalase which can ward off free radicals. Mangrove plants (*Rhizophora mucronata*) are plants that have the potential as natural antioxidants (Bulan et al., 2022). Antioxidant activity can be expressed in Inhibitory Concentration 50 (IC_{50}) (Ridlo et al., 2017). The IC_{50} value is the concentration of the extract solution which causes a reduction in DPPH activity by 50% (Usman et al., 2022).

The IC_{50} value of the tested extract was obtained from the compounds contained in the extract and fraction, where both the extract and the fraction are a collection of several secondary metabolite compounds that have not been separated (Minarti et al., 2021). Based on the potential possessed by *Rhizophora mucronata*, the objective of this research is to evaluate the content of secondary metabolite compounds contained in *R. mucronata* mangrove leaf extract and to determine its potential as a source of natural antioxidants.

MATERIALS AND METHODS

Sample preparation

Samples of *R. mucronata* mangrove leaves were obtained from the Mangrove Botanical Garden, Gunung Anyar District, Surabaya. *R. mucronata* leaves are washed and then dried manually by drying in the sun with the help of sunlight and mashed into powder. The powder sample was weighed 50 grams and extracted using n-hexane, ethyl acetate and ethanol solvents. The extraction method is carried out by putting the powder sample into the Erlenmeyer and added with n-hexane, ethyl acetate and ethanol solvents. Each solvent uses 3 Erlenmeyer and is shaking in a shaker. The filtrate obtained is then evaporated with a *vacuum rotary evaporator* with a temperature of 40°C until a crude extract is obtained.

Phytochemical analysis

Testing of compounds contained in the leaves of *Rhizophora mucronata* can be known by conducting several tests on phytochemical tests, including: Alkaloid compound tests are carried out with Mayer, Wagner and Dragendorff reagents, which if the positive results of alkaloids with Wagner and Dragendorff reagents are characterized by the presence of brown deposits on Wagner reagents and orange deposits on Dragendorff reagents. The alkaloid test is tested using Dragendorff reagents to produce a dark brown solution which means it is positive for alkaloids. The flavonoid compound test was carried out with concentrated Mg + HCl + ethanol reagent characterized by the presence of a red precipitate. The saponin test is performed without the use of reagents and is tested positive if the foam does not disappear at the time of addition of HCl. Steroid compound tests using Libermann-burchard reagents obtained positive results characterized by the presence of bluish-purple / forage deposits. Test of triterpenoid compounds using chloroform + H_2SO_4 concentrated reagent obtained positive results characterized by the presence of brownish-red precipitates. The phenolic test uses a 10% NaCl reagent + 1% gelatin and obtained positive results characterized by the presence of white precipitates. The tannin compound test using a 1% $FeCl_3$ reagent obtained positive results characterized by the presence of greenish-brown deposits.

Analysis of antioxidant activity

Antioxidant activity was measured using the method (DPPH) by making several concentrations of ethanol solvent extract and pipettes of 1 ml each, adding 3 ml of DPPH solution (40 ppm in ethanol), the solution was allowed to stand at room temperature for 30 minutes.

The manufacture of the control was carried out with 1 ml of ethanol and 3 ml of DPPH 40 ppm. The absorbance value was measured at a wavelength of 519 nm using visible light spectrophotometry.

The percentage of free radical inhibition determines antioxidant activity. Quantitative analysis of the activity of radical inhibitors or DPPH, is carried out using the following formula:

$$\% \text{ inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

After the percentage of antioxidant activity is obtained, the linear regression curve between the sample concentration and the average inhibitory percent is determined by calculating the inhibitor concentration value (IC_{50}), which is obtained from the equation $y = ax + b$ on the linear regression curve of the concentration relationship (x) and the percentage of attenuation (y).

Analysis of total phenolic content

Total phenol levels are measured using spectrophotometric methods by making a standard curve first by making a standard solution of gallic acid with a content of 5 to 40 ppm (H_2O solvent), then each pipette content of 1.0 mL put into a vial bottle and then added 0.5 mL Folin-Ciocalteu after that let stand for 5 minutes, then added 2 mL 10% sodium carbonate solution, and allowed to stand again for 10 minutes before measuring the absorbance ($\lambda = 770$ nm).

After making the standard curve is complete, proceed with sample preparation by weighing 20 mg of sample in 20 mL H_2O , then pipette 5 ml and 10 mL H_2O , then pipette again 1.0 mL aliquot and put into a vial, then added 0.5 mL Folin-Ciocalteu, then let stand 5 minutes, then added 2 mL 10% sodium carbonate solution and let stand 10 minutes before measuring the absorbance ($\lambda = 770$ nm). The test results of total phenol content on total phenolic content are expressed in milligrams of gallic acid equivalent (mg GAE/g extract) which is used as standard.

RESULTS AND DISCUSSION

Yield of extraction

Yield is a calculation of the weight of the extract compared to the initial weight of the sample used (Manuhuttu & Saimima, 2021). The calculation of the percentage of yield aims to find out the number of samples needed for extraction in order to obtain the desired amount of extract (Egra et al., 2023). The results of the extraction process in each sample are carried out through the maceration method and are presented on Table 1.

Table 1. The yield of *R. mucronata* leaves extract.

Solvent	Yield (%)
n-Hexane	0.83
Ethyl acetate	3.73
Ethanol	7.86

The yield calculation results in table 1 show that the highest yield was obtained using a polar solvent, namely

Table 2. Bioactive compounds from leaves extract of *Rhizophora mucronata*.

Bioactive compounds	Reagent	Solvent		
		Ethanol	Ethyl acetate	n-hexane
Alkaloid	Mayer	+	+	-
	Wagner	+	+	+
	Dragendorff	+	+	+
Flavonoid	Mg + HCl + etanol	+	+	+
Saponin	-	+	+	+
Steroid	Libermann-Burchard	+	+	+
Triterpenoid	Chloroform + H ₂ SO ₄ concentrated	+	+	+
Phenolic	NaCl 10% + Gelatine 1%	+	+	-
Tannin	FeCl ₃	+	+	+

Notes : (+) detected; (-) not detected

ethanol of 7.86% while the smallest yield was obtained by treatment using a non-polar solvent, n-Hexane of 0.83%. This shows that the compounds that can be extracted in *Rhizophora mucronata* mangroves are tend to be polar so as to produce the highest yield compared to non-polar solvents. This is in accordance with the research conducted by [Egra et al. \(2023\)](#) which depicted that extraction of *R. mucronata* leaves using n-Hexane solvent yielded the smallest yield of 0.19% compared to ethyl acetate solvent of 1.87%. According to [Maulana & Sasmito \(2021\)](#), extraction of *Rhizophora apiculata* using ethanol solvent (5.91%) produces the highest yield of ethyl acetate (1.31%) and n-hexane (1.18%) solvents, indicating that the compounds contained in mangrove leaves tend to be polar.

The yield percentage is influenced by the bioactive content contained in the material and the type of solvent used. During maceration, the cell wall and cell membrane will rupture due to the pressure difference between the outer and inner cells. Secondary metabolites are released in the cytoplasm and dissolved in the solvent ([Li et al., 2019](#)). Based on [Hardiningtyas et al. \(2014\)](#), The selection of solvents based on their solubility and polarity will affect the extraction yield because solvents, the ease of separation of natural materials in the sample depends on the type of solvent and the bioactive content contained in the sample is related to the amount of yield value produced. Different yield percentages are also due to different physiological adaptation patterns to the environment. [Yang et al. \(2018\)](#) stated that bioactive compounds that plants are responding to extreme environmental conditions especially light irradiation, temperature, nutrient supply, salinity, etc.

The color of n-hexane extract was brownish yellow, while the ethyl acetate and ethanol extract were dark green. According to [Indriaty et al. \(2023\)](#) stated that *Rhizophora* mangrove extracts generally have a solid form and are reddish-brown in color. Reddish-brown color indicates the presence of tannins, which have chromophore groups in the conjugated C=C and C=O bonds, which absorb and produce color in a compound. In addition, there are also plant pigments that affect other colors in Rhizophoraceae extracts derived from chlorophyll a, chlorophyll b, beta carotene, lutein, neoxanthin, pheophytin a, and violaxanthin ([Rumengen et al., 2021](#))

Phytochemical testing

Phytochemical tests in this study aim to identify secondary metabolites produced by plants ([Egra et al., 2023](#)). The phytochemical testing of this study includes assays of alkaloids, flavonoids, saponins, steroids, triterpenoids, phenolics, and tannins. Based on the results of phytochemical tests in [Table 2](#), it shows that all *R. mucronata* mangrove leaf extracts contain secondary metabolites such as flavonoids, saponins, steroids, triterpenoids, and tannins. Alkaloid and phenolic compounds were found in ethanol and ethyl acetate extracts of *R. mucronata* leaves, but not in n-Hexane extracts of *R. mucronata* leaves. In previous studies, phytochemical screening of *R. mucronata* ethanol extract identified the presence of alkaloids, flavonoids, tannins, saponins and phenolics ([Mile et al., 2021](#)). Ethyl acetate extract of *R. mucronata* mangr ayer's reagent, the formation of brown deposits in Wagner's reagent and the formation of orange deposits in Dragendorff reagents ([Aji et al., 2023](#)). Alkaloids are not found in n-hexane extracts of *R. mucronata* leaves allegedly because n-hexane solvents are nonpolar solvents unable to dissolve alkaloid compounds. This is in accordance with the statement [Manteu et al. \(2018\)](#), that Alkaloids are chemical compounds that can dissolve in organic solvents and are widely found in extracts that use polar solvents.

Flavonoids showed positive reactions in all *R. mucronata* leaves extracts characterized by the formation of red color when reacted with concentrated Mg + HCl + ethanol reagents. Flavonoids act as antioxidants because they can capture reactive oxygen species (ROS) and prevent ROS regeneration while indirectly increasing enzymatic and cellular antioxidants. Prevention of ROS formation by flavonoids is done in several ways, such as inhibiting the enzymes xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) and preventing redox reactions that can produce free radicals ([Liu et al., 2018](#)).

Other metabolic compounds detected in the leaves of *Rhizophora mucronata* are saponins, steroids, triterpenoids, and tannins. Samples that tested positive for saponin compounds were characterized by foam that did not disappear upon addition of HCl ([Sari et al., 2019](#)). Saponins can dampen superoxide through hydroperoxide intermediates, which prevent

biomolecular damage due to free radicals (Liu et al., 2018). *R. mucronata* leaves containing steroid compounds are evidenced by the formation of purple to blue / green colors. According to Egra et al. (2023), *R. mucronata* leaf extract has a high steroid content, while alkaloids, saponins, tannins, phenols, and flavonoids tend to be low. Steroids can increase body stamina (aphrodisiac) and act as anti-inflammatory (Permana et al., 2016). The detection of triterpenoid compounds is thought to be caused by the ability of ethanol solvents to extract polar compounds (Dia et al., 2015). The positive reaction of triterpenoids in the sample is indicated by the formation of a reddish-brown color (Manteu et al., 2018).

The tannin compound test using a 1% FeCl_3 reagent obtained positive results as evidenced by the formation of a brownish-yellow color due to a solution of tannin compounds containing many OH groups in the solvent, both of which are polar (Acacia et al., 2021). Tannins as antioxidants can act as H atom donors to dampen DPPH radicals, which occur as tannin radical stabilizers due to resonance (Liu et al., 2018). Tannins have several properties, such as astringent, anti-diarrheal, antibacterial, and antioxidant. Flavonoids have anti-inflammatory, antibacterial and antioxidant properties (Egra et al., 2023).

Phytochemical testing with n-hexane solvent gave positive results of *R. mucronata* mangrove leaves not positive for phenolic compounds while phytochemical testing using ethyl acetate and ethanol solvents showed that *R. mucronata* mangrove leaves were positive for phenolic compounds. The use of solvents that have different polarity levels can be one of the factors for the non-detection of phenolic compounds in extracts that use nonpolar solvents such as n-hexane. Phenolic compounds contained in the leaves of *R. mucronata* are characterized by the presence of white precipitate using NaCl 10% + gelatin 1%. The research conducted by (Syamsudin et al., 2022) revealed that a different final result, namely the color of the sample becomes blue-black because it uses a 5% FeCl_3 reagent. The use of solvents with increased or high polarity aims so that each solvent can dissolve compounds based on their level of polarity (Haryoto & Frista, 2019).

Antioxidant activity

The body needs compounds known as antioxidants to stop oxidation reactions consisting of free radicals (Loho et al., 2021). The DPPH test is widely used to evaluate the potential of plant extracts to neutralize free radicals, especially organic radicals. The ability of the extract or vitamin C (as a positive control) to donate hydrogen electrons and bind with the nitrogen radical in DPPH causes the purple color solution of the DPPH radical to turn light yellow (Bulan et al., 2022). The ability of the extract to inhibit antioxidants is determined based on the value of IC_{50} (Gazali et al., 2020). This value indicates the sample concentration required to reduce free radical activity. Test of antioxidant activity of *R. mucronata* leaf extract showed that DPPH solution turned yellow, from the previous concentrated ungu. Based on Table 3, the ethanol, ethyl acetate, and n-hexane extracts of *R. mucronata* leaves showed high antioxidant activity.

This is in accordance with the statement Leksono et al. (2018) which indicated that a compound is considered a very strong antioxidant if its IC_{50} value is less than 50 ppm ($\text{IC}_{50} < 50 \text{ ppm}$), strong ($50 \text{ ppm} < \text{IC}_{50} < 100 \text{ ppm}$), medium ($100 \text{ ppm} < \text{IC}_{50} < 150 \text{ ppm}$), and very weak ($\text{IC}_{50} > 200 \text{ ppm}$).

Table 3. The IC_{50} value of *R. mucronata* leaves extract.

Solvent	IC_{50} value
n-hexane	$4.90 \text{ mg} \pm 0.82\%$
Ethyl acetate	$1.70 \text{ mg} \pm 0.17\%$
Ethanol	$0.21 \text{ mg} \pm 1.90\%$

Antioxidant activity in samples with ethanol solvent had the highest antioxidant levels with the smallest IC_{50} value compared to other solvents at $0.21 \text{ mg} \pm 1.9\%$. Inhibitory Concentration (IC_{50}), which is the concentration of an antioxidant that stops free radical activity by 50% (Leksono et al., 2018). If the resulting IC_{50} value is lower, it indicates that the ability of antioxidant activity is getting better (Filbert et al., 2014). According to Rumengen et al. (2021) states that the leaf extract of *Rhizophora mucronata* has very strong antioxidant activity. The IC_{50} value of ethanol extract of *Rhizophora mucronata* leaves from Lembeh Island, North Sulawesi, Indonesia with DPPH method showed $\text{IC}_{50} 20.99 \pm 0.33 \text{ } \mu\text{g/mL}$. *R. mucronata* leaf extract has antioxidant activity because hydrogen atoms or electrons are donated to react with DPPH radicals. Increased extract concentration also increases the percentage of DPPH free radical inhibition (Rumengen et al., 2021). This suggests that *R. mucronata* leaf extract has strong scavenging abilities in its role as a hydrogen atom donor.

The antioxidant ability of mangrove plants is influenced by the content of secondary metabolites, which function as antioxidant supporting compounds, such as tannins, phenolics, and flavonoids (Moito et al., 2023). The higher the amount of phenol compounds and flavonoid compounds in a sample will provide higher antioxidant activity so that the IC_{50} value will be lower (Safithri et al., 2020). This is proven in phytochemical tests of n-hexane extract showing fewer secondary metabolite compounds hence that the antioxidant ability possessed is smaller than ethanol and ethyl acetate extracts. The difference in metabolite content in mangroves is caused by differences in age, geographical location and climate which will affect the content of bioactive compounds in mangrove plants. Extreme environmental conditions (especially light irradiation, temperature, nutrient supply, salinity, etc.), plants are able to adapt to changing circumstances through the release and accumulation of various secondary metabolites including phenolic compounds, triterpenoids and flavonoids, and many of these have high economic value and utilization due to antioxidant properties (Yang et al., 2018).

Total phenolic content

Total phenol testing is usually used to determine the content or antioxidant activity of a sample used. One sample that can be used to determine the content or activity of antioxidants is usually found in the leaves of the mangrove *Rhizophora mucronata* which is known as

one of the good sources of antioxidant production. Total phenol is the main component that is closely related to antioxidant activity which further to obtain the total phenolic concentration in the extract can be determined by testing using Folin-Ciocalteu reagent (Sopalun *et al.*, 2021).

Table 4. Total phenolic content from *Rhizophora mucronata* leaves extracts.

Solvent	Total Phenolic Content
n-hexane	0.04% w/w ± 0.01%
Ethyl acetate	0.09% w/w ± 0.01%
Ethanol	6.70% w/w ± 0.02%

Table 4 depicted that the total phenol content is different for each solvent. The highest total phenol content was obtained from extraction with ethanol, which was 6.7% w/b ± 0.02%. The lowest total phenol content was obtained from extraction with n-hexane of 0.04% w/b ± 0.01%. According to research conducted by Permana *et al.* (2016), The presence of phenol compounds in a material indicates the presence of antioxidant content in the material. Research by Prasedya *et al.* (2019) indicates that higher phenol content indicates stronger antioxidant activity, as evidenced by lower IC_{50} values. It is proven that the antioxidant content of *R. mucronata* ethanol extract is higher because it contains the highest total phenol. Ethanol is a solvent that can dissolve compounds from less polar to polar, one of the compounds that can be dissolved by ethanol is phenolic compounds. Ethanol can dissolve phenolic compounds because it is able to degrade the cell wall so that bioactive compounds more easily come out of plant cells. Ethanol has a hydroxyl group that can bond to the hydrogen group of the hydroxyl group of the phenolic compound leading to an increase in the solubility of phenolic compounds in ethanol. Differences in ethanol concentration can affect the solubility of phenolic compounds in solvents (Prayitno *et al.*, 2016).

Total phenols in *R. mucronata* have previously been studied by Hastuti *et al.* (2023) which states that the total phenolic leaves of *R. mucronata* in Tugu District, Semarang, Central Java 3.90 ± 1.11 (%w/w). Moreover, Suhendra *et al.* (2019), stated that ethanol concentration affects total phenol levels, yield, total flavonoids and DPPH radical inhibitor activity which also produces the highest content obtained from 70% ethanol extract. Maria *et al.* (2020) states that 48.2% phenol content is present in *R. mucronata*. The total phenolic content is expressed in milligrams of gallic acid equivalent (mg GAE/g extract) used as standard. Phenolic compounds such as flavonoids can act as antioxidants because they contain hydroxyl groups bonded to the aromatic carbon ring so that they can capture free radicals (Saxena *et al.*, 2013). Phenolic compounds can react with free radicals because they have the ability to donate electrons (reducing agents) to produce more stable products and inhibit free radical chain reactions (Plaza *et al.*, 2014).

CONCLUSIONS AND RECOMMENDATION

Conclusions

The results of antioxidant tests show that *R. mucronata* mangroves can function as natural antioxidants because of the content of secondary metabolites and phenolic compounds in the leaves. The compounds that can be extracted in the leaves of *Rhizophora mucronata* mangroves are semi-polar because they produce the highest yield (0.0748%) in ethyl acetate extract. Phenolic compounds contained in all mangrove leaf extracts (*Rhizophora mucronata*) include flavonoids, saponins, steroids, triterpenoids, and tannins with total phenols ranging from 0.04-6.7%. The magnitude of antioxidant activity, *R. mucronata* is included in the strong antioxidant because the IC_{50} value in each sample was $4.9 \text{ mg} \pm 0.82\%$ in n-Hexane extract, $1.7 \text{ mg} \pm 0.17\%$ in ethyl acetate extract, and $0.21 \text{ mg} \pm 1.9\%$ in ethanol extract.

Recommendation

Research on antioxidant activity and phytochemical screening of *Rhizophora mucronata* mangrove leaf extract needs further research, especially related to the characterization of specific compounds that act as a of antioxidants. In addition, potential bioactive compounds function as antioxidant could be further developed as natural preservatives in food commodities or therapeutic agents.

AUTHORS' CONTRIBUTIONS

In preparing this article, RR, YAS, YSP, EPNH, and ZNS contributed in several activities, starting from conducting research activities to preparing manuscript, while TA specifically involved in data analysis and preparing manuscript.

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