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Characterization and Comparative Assessment of the Essential Oil from Lime (*Citrus aurantifolia*) Exocarp Using Maceration and Soxhlet Extraction Methods

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Abstract

Citrus fruits are a rich source of essential oils that have various applications in the cosmetic, food, and pharmaceutical industries. Lime is notable for its high essential oil yield, which contains active compounds that possess antimicrobial, antioxidant, and anticancer properties. This study aimed to compare the maceration and Soxhlet extraction methods for obtaining essential oil from lime exocarp as well as characterizing the compounds in the oil using Gas Chromatography-Mass Spectrometry (GC-MS). The study found that the Soxhlet extraction method had a higher yield of oil compared to the maceration method. However, the maceration method had a lower acid value and free fatty acid content, and a higher saponification value. The oil obtained using the Soxhlet extraction method was more acidic than that of the essential oil obtained using the maceration method. The study also found that limonene was the most prominent compound in both extraction methods. However, the percentage of β-ocimene and γ-terpinene were significantly higher in the maceration method compared to the Soxhlet extraction method. Limonene, β-ocimene, and γ-terpinene are important compounds found in essential oils and have various medicinal properties. These findings have significant implications for the essential oil industry. The choice of extraction method can influence the composition of the essential oil obtained, as well as its chemical and physical properties. Therefore, it is crucial to consider the intended use of the essential oil when choosing an extraction method.

Keywords: Lime Exocarp, C*itrus aurantifolia*, Essential oil, Yield, Maceration method, Soxhlet extraction methods, Characterization.

INTRODUCTION

Citrus fruits are widely recognized as important sources of essential oils that have various applications in the cosmetic, food, and pharmaceutical industries (Taheri *et al.*, 2015; Oderinde *et al.*, 2019). Among the citrus fruits, lime (*Citrus aurantifolia*) is notable for its high essential oil yield, which contains active compounds such as limonene, citral, linalool, and terpineol that possess antimicrobial, antioxidant, and anticancer properties (Ozcan and Chalchat, 2002; Bentayeb *et al.*, 2017). Lime essential oil is usually extracted from the exocarp or outer layer of the fruit using various methods such as steam distillation, solvent extraction, and cold pressing (Misran *et al.*, 2020). However, the choice of extraction method can affect the quality and quantity of the essential oil obtained. Previous studies have shown that the essential oil yield and chemical composition of lime exocarp varies with extraction methods and conditions. For example, Ozcan and Chalchat (2002) reported that the yield of lime essential oil obtained by hydrodistillation was 0.23% and 0.43% for fresh and dried exocarp, respectively.

Similarly, Misran *et al.*, (2020) found that steam distillation and Soxhlet extraction methods gave different yields and chemical profiles of lime essential oil from Malaysian limes. However, there is limited research on the comparative assessment of the maceration and Soxhlet extraction methods for lime essential oil production. Therefore, a comparative assessment of the essential oil yield from lime exocarp using maceration and Soxhlet extraction methods is necessary to determine the most efficient and cost-effective method for obtaining high-quality oil. The study also identified the most efficient and cost effective method for the production of high-quality lime essential oil.

MATERIALS AND METHOD

Research Design

The comparative assessment of essential oil yield from lime (*Citrus aurantifolia*) exocarp using maceration and Soxhlet extraction methods was carried out in accordance with the procedures described by previous studies (Azevedo-Meleiro and Rodriguez-Amaya, 2004; Leite *et al.*, 2008). To obtain the exocarp of the fruit, matured limes were carefully peeled off, washed with water, and air-dried until it reaches a constant weight. The powdered exocarp was then subjected to maceration and Soxhlet extraction for each method. The obtained oil yield from each extraction method was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify the chemical components present in the essential oil. The analysis was done in accordance with the standard protocol, and the data obtained was recorded. The data collected were analyzed using statistical software to perform a comparative assessment of the yield from both extraction methods.

Collection of Lime Exocarp

Matured fruits of lime (*Citrus aurantifolia*) were purchased from a local market in the Port Metropolis in Rivers State, Nigeria. The lime was botanically identified using the standard morphological characteristic features. Limes were bought in plastic bags and were washed with water.

Figure 1: (a) Matured, Fresh and Unpeeled Lime (*Citrus aurantifolia*); (b) Lime Exocarps

Preparation of Lime Exocarp

The exocarps were carefully peeled off from the fruit using a clean knife. Thereafter, the lime exocarps were placed in a clean sac, spread out, and air-dried at room temperature until it reaches a constant weight for two weeks. The air-dried lime exocarps were then grinded to give a consistent and fine powder using an Electric Blender. The powdered peels were then stored in an airtight container at ambient temperature and protected from sunlight for further use in the Biochemistry Laboratory in the Department of Biochemistry, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, for extraction and characterization of oil.

Figure 2: Dried Blended Lime Exocarp

Analytical methods to measure the constants of fats and oils

Soxhlet Extraction Method

40g of grinded lime exocarp was measured into a thimble, sealed and placed in the sample chamber of the Soxhlet Apparatus. This was then extracted with 100ml of hexane continuously for 3 hours at 60 degrees Celsius. The solvent was recovered and the oil placed in a beaker over a water bath for 1 hour. The oil was then weighed and used to calculate the yield. Oil quality parameters were then determined.

Figure 3: (a) Maceration method; (b) Soxhlet Extraction Apparatus

Maceration Method

40g of lime exocarp sample was measured into a conical flask and 100 ml of hexane was added. This was agitated and sealed. The sample was shaken intermittently for 3 days and then filtered. The solvent was then evaporated. The recovered oil was dried in a beaker over a water bath for 1 hour, weighed, and used to determine the oil quality parameter.

Acid value (Acid number) determination

The acid value (AV) is the number that expresses (in milligrams) the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, e.g., amino acids or acid phosphates. The acid value is often a good measure of the breakdown of the triacylglycerols into free fatty acids, which has an adverse effect on the quality of many lipids. Hence, the acid value is the measure of hydrolytic rancidity. In general, it gives an indication of the edibility of the lipid. Edible oil contains >1% hydrolytic rancidity and pharmaceutical oil must not have any acidity.

Materials: Oil, Absolute ethanol alcohol, Phenolphthalein and 0.1 N KOH

Procedure: 1g of oil was placed in a dried conical flask. 5 ml of absolute ethanol alcohol was added to the oil and (2-3) drops of phenolphthalein were added too. It was heated while shaking in a water bath (65%) for 10 minutes, then cooled. Then the solution was titrated against 0.1 N KOH until pink color appeared (endpoint). Observations was recorded. The acid value (AV) and free fatty acid (%FFA) was calculated using the formula below;

$$AV = \frac{ml\ of\ KOH\ x\ N\ x\ 56}{Weight\ of\ Sample} = mg\ of\ KOH$$

Where; N = Normality of KOH

$$\%\ Free\ Fatty\ Acid\ (FFA) = AV\ x\ 0.503$$

Saponification Number

The saponification value is the number of milligram of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length).

Materials: Oil, 0.5N alcoholic potassium hydroxide (alcoholic KOH) (prepared by dissolving 30 g potassium hydroxide in 20 mL of water and make the final volume to 1 L using 95 % ethanol. Then the solution was left to stand for 24 hours before decanting and filtering the solution; 0.5N Hydrochloric acid, Phenolphthalein.

Procedure: Approximately 2 g of the oil was weighed and put into a 250 mL conical flask. 25 mL of alcoholic potassium hydroxide solution (0.5N) was added. A reflux condenser was attached and the flask contents was heated on a boiling water bath for 1 hour with occasional shaking. While the solution was still hot, 3

drops of phenolphthalein indicator was added and the excess potassium hydroxide was titrated with the 0.5N hydrochloric acid (Vml of hydrochloric acid at end point represents S). The same procedure was repeated but without sample (Vml of hydrochloric acid at end point represents B). Saponification number was calculated by using the formula below;

$$SP = \frac{56.1\ (B - S)x\ N\ x\ HCL}{Gram\ of\ Sample}$$

Where;

B = ml of HCl required by Blank.
S = ml of HCl required by Sample

Iodine Value (I.V)

The iodine value (I.V) gives a measure of the average degree of unsaturation of a lipid. The higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid. Iodine value (I.V) is directly proportional to the degree of unsaturation (No of double bonds.) and inversely proportional to the melting point (M.P) of lipid. An increase in I.V indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation. One of the most commonly used methods for determining the iodine value of lipids is "Hanus method". The lipid to be analyzed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine chloride is added. Some of the IBr reacts with the double bonds in the unsaturated lipids, while the rest remains:

$$R - CH = CH - R + IBr_{excess} = R - CHI - CHBr - R + IBr_{remaining}$$

The amount of IBr that has reacted is determined by measuring the amount of IBr remaining after the reaction has gone to completion ($IBr_{reacted} = IBr_{excess} - IBr_{remaining}$). The amount of IBr remaining is determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating with a sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released

$$IBr_{remaining} + 2KI = KBr + KI + I_2$$
$$I_2 + Starch + 2Na_2S_2O_3(Blue) = 2Nal + Starch + 2Na_2S_4O_6(Colourless)$$

Materials: Oil, Hanus solution (it is prepared by dissolving 18.2 g of iodine in 1L of glacial acetic acid and then adding 3 ml of bromine water for increasing the halogen content, 15% potassium iodide solution, 1% starch solution, 0.1 N Sodium thiosulfate solution.

Procedure: Approximately 0.25 g of the oil was weighed and put into a 250 mL conical flask. 10 ml of chloroform was added. 30 ml of Hanus solution was added and the flask was closed completely by Para film, then the solution was left for 30 minutes while shaking continuously. 10 ml of 15% potassium iodide solution was added and then shaken. 100 ml of distilled water (DW) was added. Iodine solution was titrated against 0.1 N Sodium thiosulfate solution till a yellow color formed, then 2-3 drops of the starch solution was added and a blue solution formed, titration was continued till the blue colour disappeared (Volume (ml) of $Na_2S_2O_3$ at endpoint represents S) Same above procedure was repeated but without a sample (Volume (ml) of $Na_2S_2O_3$ at endpoint represents B). The iodine number was calculated by using the formula below;

$$Iodine\ number = \frac{(B - S)\ x\ N\ of\ 2Na_2S_2O_3\ x\frac{0.127g}{meg}x\ 100\ Iodine\ value}{Weight\ of\ sample\ (g)}$$

Where;

B = V ml of $Na_2S_2O_3$ volume for blank
S = V ml of $Na_2S_2O_3$ volume for sample

Physical characteristics of the extraction of essential oil

This was carried out by sensory analysis of the essential oil to determine its physical properties which are smell, sight, and solubility.

Determination of the solubility of the essential oil

A few drops of oil were added to the test tube containing a little amount of water. The test tube was stirred thoroughly with a stirring stick.

Essential oil analysis using GC-MS

The obtained oil yield from both extraction methods was subjected to GC-MS analysis to determine the chemical composition of the essential oil. The GC-MS analysis method as described by Zhang et al. (2021) was followed.

Material/Instruments

All glassware used in the extraction of essential oil were washed with dilute nitric acid and rinsed in distilled water, and dried in a hot oven prior to use, to avoid any unwanted reaction during the extraction process.

Data Analysis

Data obtained were analyzed using Statistical Package for Social Science (IBM SPSS) version 25.0. Descriptive statistics to get the mean value of data was carried out, and inferential statistics using one-way analysis of variance (ANOVA) was done to check for significance difference at 95% ($p<0.05$) confidence interval between the groups.

RESULTS

Figure 4 and Table 1 show the appearance and physical properties of the oil from maceration and Soxhlet extraction methods of lime exocarp. The essential oil obtained from both extraction methods was dark green in color with a citrusy small for the essential oil extracted using the maceration method and a tangy smell for the essential oil extracted using the Soxhlet extraction method. The oil extracted by both methods was observed to be insoluble in water. Table 2 compares the chemical properties of the oil extracted from lime exocarp using maceration and Soxhlet extraction methods. The results show that the Maceration method has a lower acid value and free fatty acid content but a higher saponification value. The Soxhlet Extraction method has a higher iodine value compared to the maceration method.

Table 3 shows a comparison between the maceration and Soxhlet extraction methods for obtaining oil from lime exocarp. The weight of oil obtained was significantly ($p<0.05$) higher in the Soxhlet extraction method compared to maceration. The percent yield of oil was also significantly higher for Soxhlet extraction compared to Maceration at $p<0.05$. Table 4 compares the relative percentages of different compounds in the essential oil from Lime extracted using maceration and Soxhlet extraction methods. The most prominent compound in both methods was limonene, with 48.78% and 69.2% for maceration and Soxhlet extraction respectively. The percentage of β-ocimene was significantly higher in maceration (16.68%) compared to Soxhlet extraction (4.6%). Similarly, the percentage of γ-terpinene was higher in maceration (8.43%) compared to Soxhlet extraction (5.4%). Other compounds in the essential oil include β-pinene, α-pinene, sabinene, myrcene, terpinolene, α-terpinene, caryophyllene, and nonterpenoids.

Figure 4: (a) Lime essential oil extracted using maceration method; (b) Lime essential oil extracted using Soxhlet extraction method

Table 1: Comparison of the Physical Properties of the oil from Maceration and Soxhlet Extraction methods of Lime Exocarp

Physical Properties	Maceration method	Soxhlet Extraction method
Colour	Dark Green	Dark Green
Smell	Citrusy	Tangy
Solubility	Insoluble in water	Insoluble in water

Table 2: Comparison of the Chemical Properties of the oil from Maceration and Soxhlet Extraction methods of Lime Exocarp

Quality Parameters	Maceration method	Soxhlet Extraction method
Acid value (mg KOH/g oil)	7.18	10.24
% Free fatty acid	3.12	5.17
Saponification value (mg KOH/g oil)	130.37	116.65
Iodine value (g I_2/100g oil)	91.02	98.43

Table 3: Comparison of Maceration and Soxhlet Extraction methods of Lime Exocarp

Parameters	First extraction	Second Extraction	p-value
Weight of ground sample	40g	40g	-
Volume of solvent	100 ml	100 ml	-
Weight of oil (Maceration)	2.64g	2.62g	**0.000***
Weight of oil (Soxhlet)	6.35g	6.36g	
% Yield (Maceration)	6.60%	6.55%	**0.000***
% Yield (Soxhlet)	15.88%	15.90%	

*significant difference at p<0.05

Table 4: The chemical composition of the essential oil from Lime (*Citrus aurantifolia*) exocarp extracted using maceration and Soxhlet extraction methods, as analyzed through GC-MS analysis

Compound Name	Maceration method	Soxhlet extraction method
	Relative Percentage (%)	Relative Percentage (%)
limonene	48.78	69.2
β-ocimene	16.68	4.6
γ-terpinene	8.43	5.4
β-pinene	7.08	5.8
α-pinene	5.67	2.1
sabinene	5.06	1.7
Myrcene	2.86	2.4
terpinolene	1.89	3.8
α-terpinene	1.32	0.4
caryophyllene	1.13	0.2
Nonterpenoids	0.38	0.8

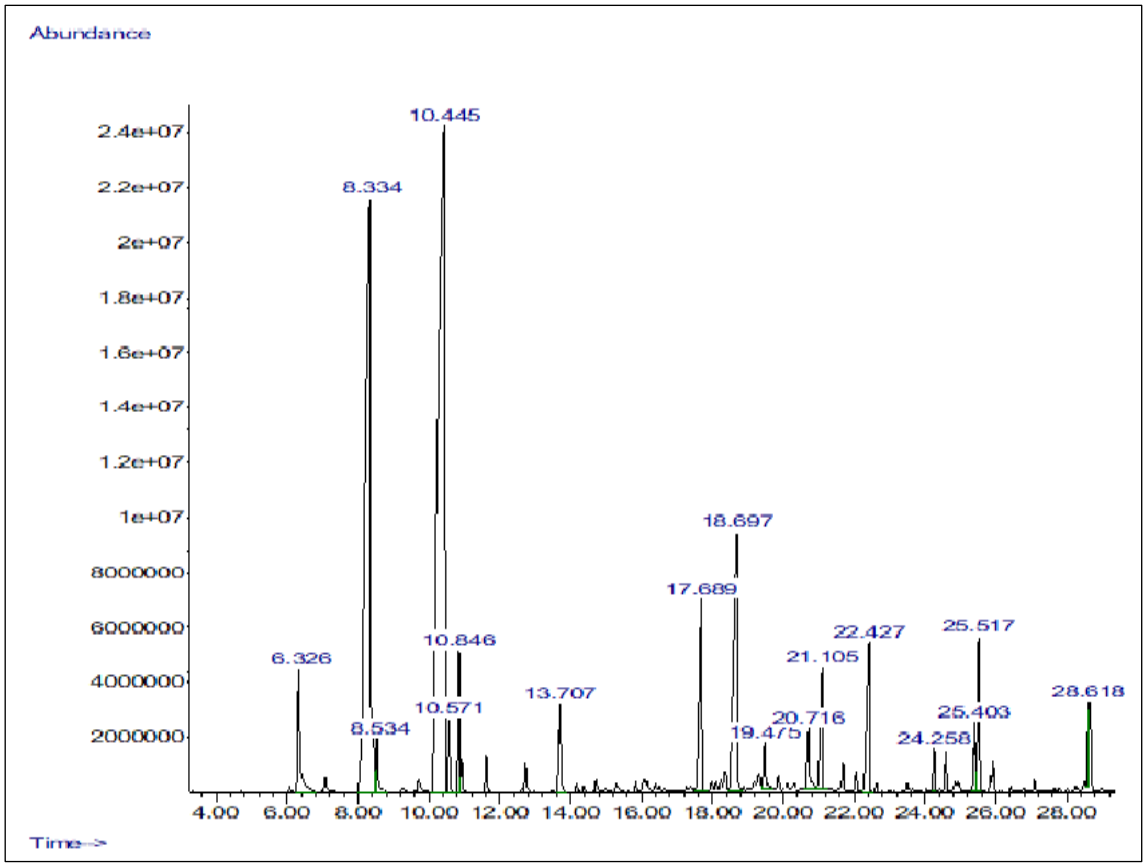

Figure 5: GC-MS of lime exocarp essential oil (*C. aurantifolia*)

DISCUSSION

The present study aimed to compare the maceration and Soxhlet extraction methods for obtaining essential oil from lime exocarp as well as characterizing the compounds in the oil using GC-MS. The physical properties of the oil obtained by both methods were found to be similar, but the smell was different. The maceration method employs a solvent to drain the scent of an ingredient. The ingredient being utilized is soaked in the solvent for a specific amount of time, which is typically several days or weeks in order to achieve an ideal scent profile. On the other hand, the Soxhlet method is a distillation process that aims to concentrate the scent by boiling it and removing the moisture from it. As a result, the two processes will result in different scent profiles since the maceration process draws out a more nuanced array of scents, while the Soxhlet method can produce a more concentrated and stronger scent (Braga *et al.,* 2012; Siddiqui *et al.*, 2013). Maceration method may yield scents that are more similar to the natural scent of the ingredient, while Soxhlet may yield scents that are more potent and powerful (Ratky and Noma, 2008; Baggio *et al.,* 2015), and according to Andrade *et al.* (2008) and Ellong *et al.* (2013), maceration method may also take longer to yield extracts compared to other processes such as distillation and steam distillation.

Additionally, the chemical properties of the oil extracted using the two methods differed significantly. This agrees with the report of Mbonu *et al.* (2012). These differences in chemical properties can also be attributed to the difference in the extraction process (Kulkarni and Chidambara, 2017). The maceration method allows for a slower and gentler extraction process, which may result in a higher concentration of certain compounds and a different chemical profile, while the repeated cycles of boiling and condensation in Soxhlet extraction may cause changes in the chemical composition of the oil, such as degradation of temperature-sensitive compounds (Kulkarni and Chidambara, 2017). The higher temperature and pressure may also alter the solubility of certain compounds, resulting in a different chemical profile than the maceration method (Anjum *et al.*, 2018; Wang *et al.*, 2020). Hence, the Soxhlet extraction method is a more aggressive extraction process compared to the maceration method.

According to the study, the Soxhlet extraction method had a higher yield of oil compared to the maceration method. However, the maceration method had a lower acid value and free fatty acid content, and a higher saponification value. The oil obtained using the Soxhlet extraction method was more acidic than that of the essential oil obtained using the maceration method. The higher the acid level, the faster the deterioration of the essential oil. Oils with lower acid levels are safer for making skincare products (Calixto, 2005; Ognimba *et al.,* 2021). Essential oils are concentrated and they contain many volatile aromatic compounds which are mostly free fatty acids (Roberty and Rodney, 2014). Free fatty acids are responsible for oil rancidity, the lesser the free fatty acid value, the lesser the rancidity of the oil (Roberty and Rodney, 2014; Kadam *et al.,* 2019). The higher the saponification value, the lower the free fatty acids (Roberty and Rodney, 2014; Kadam *et al.,* 2019). The saponification value is an indicator of the average molecular weight and the average length of free fatty acids (Roberty and Rodney, 2014). The iodine value indicates the degree of unsaturation of fat and oil (Roberty and Rodney, 2014; Kadam *et al.*, 2019). The greater the iodine value, the more unsaturation, and susceptibility to oxidation (Kadam *et al.*, 2019). These differences in chemical properties indicate that the two extraction methods may result in different compositions of essential oil. The study also found that limonene was the most prominent compound in both extraction methods. However, the percentage of β-ocimene and γ-terpinene were significantly higher in the maceration method compared to the Soxhlet extraction method. This is similar to report by Yang *et al.* (2021).

Limonene, β-ocimene, and γ-terpinene are important compounds found in essential oils and have various medicinal properties. Limonene has been shown to possess anti-inflammatory, anti-carcinogenic, and antitumor activities (Puupponen-Pimiä *et al.*, 2001; Murakami *et al.,* 2005). It is also known to have a calming effect on the mind and body and can be used in aromatherapy to reduce stress and anxiety (Sakurai *et al.,* 2002). β-ocimene exhibits antifungal and insecticidal properties and has been used as a natural insect repellent (Bakkali *et al.*, 2008; Kim *et al*., 2008). It is commonly found in citrus fruits (such as lime), mint, and basil essential oils and is used in the fragrance industry (Bakkali *et al.,* 2008). γ-terpinene has been reported to have antifungal, antimicrobial, and anti-inflammatory effects (Dorman and Deans, 2000; Alves-Silva *et al.,* 2013). It is found in various essential oils, including tea tree, eucalyptus, and thyme oils, and has been used in traditional medicine to treat respiratory disorders (Dorman and Deans, 2000). Overall, limonene, β-ocimene, and γ-terpinene are important compounds in essential oils that possess various medicinal properties and are used in aromatherapy, natural insect repellents, and traditional medicine. Other compounds identified in the essential oil extracted from Lime exocarp using both methods included β-pinene, α-pinene, sabinene, myrcene, terpinolene, α-terpinene, caryophyllene, and nonterpenoids. These findings have significant implications for the essential oil industry. The choice of extraction method can influence the composition of the essential oil obtained, as well as its chemical and physical properties. Therefore, it is crucial to consider the intended use of the essential oil when choosing an extraction method.

CONCLUSION

The study comparing the maceration and Soxhlet extraction methods for obtaining essential oil from lime exocarp found significant differences in the chemical properties of the oil, with the maceration method resulting in a lower acid value, a lower free fatty acid content, and a higher saponification value. The two methods also resulted in different compositions of essential oil, with the maceration method producing a higher percentage of β-ocimene and γ-terpinene. These findings highlight the importance of choosing the appropriate extraction method for obtaining essential oils with desired chemical and physical properties.

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