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EXTRACTION, PHYSICOCHEMICAL PROPERTIES AND ANTI-MICROBIAL ACTIVITIES OF OIL FROM *CITRUS SINENSIS* (ORANGE) AND *MAESOBOTRYA BARTERI* (BUSH CHERRY) SEEDS

***[1,2]Babatolu, A. Oluwaseun, [3]Akinsuli, V. Olawale, [1]Adekunbi, E. Adedayo, [1]Okoro, O. Sam, [4]Dada, S. Fatima and [4]Babatolu, O. Ibironke**

[1]Department of Chemistry, Adeyemi Federal University of Education, Ondo, Ondo State, Nigeria
[2]Department of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria
[3]Department of Chemistry, Federal University of Technology, Owerri, Imo State, Nigeria
[4]Department of Home Economics, College of Education, Zuba, Abuja, Nigeria

*Corresponding authors' email: babatoluayotunde@gmail.com Phone: +2348067167335

ABSTRACT

Oil from seeds of *Citrus sinensis* and *M. barteri* were extracted by solvent extraction using n-hexane. The percentage yield of the oil from seeds of *Citrus sinensis* and *M. barteri* was ascertained as 36.21 % and 12.56 % respectively. Physicochemical properties of the seed oils of *Citrus sinensis* and *M. barteri* were determined using standard methods. The extracted oils were further examined for antimicrobial studies. Nonetheless, oil extracted from *Citrus sinensis* and *M. barteri* seeds exhibits notable antimicrobial activity against every tested organism, including fungi and bacteria. However, at a concentration of 200 mg/ml, the oil from *Citrus sinensis* seeds showed more remarkable and excellent antimicrobial activity against *E. coli*, *S. typhi*, and *C. albicans* than the oil from *M. barteri* seeds. Therefore, Utilization of these seeds for residential and industrial usage in cosmetics and pharmaceutical will reduce environmental wastes and enrich their commercial value. Additionally, they offer legitimate channels for the pharmaceutical and cosmetic industries to channel these underutilized bio-resources (seeds) into medicines, soaps, and creams.

Keywords: Antimicrobial activity, *Citrus sinensis*, Extraction, *Maesobotrya barteri*, Physicochemical properties

INTRODUCTION

Citrus sinensis is a member of the Rutaceae family and the Aurantioideae subfamily. It is grown extensively in Nigeria and many other tropical and subtropical regions (Bovili, 1996; Piccinelli *et al.*, 2008; Atolani *et al.*, 2012; Jorge *et al.*, 2016). Orange, also known as the sweet orange (Citrus sinensis L. Osbeck), is a major source of vitamins, particularly vitamin C, as well as a sufficient amount of folacin, Calcium, Potassium, Thiamine, Niacin, and Magnesium (Angew, 2007). Due to its high nutritional value and other uses, citrus flavonoids have been reported to have biological action and health effects as antioxidants (Etebu & Nwauzoma, 2014; Tripoli *et. al.*, 2007). Orange waste/trash has yielded a number of value-added products, including ethanol, hesperidin, and nanocellulose (Cypriano *et al.*, 2018). According to reports, a variety of seeds have antimicrobial properties (Atolani *et al.*, 2019a, 2019b).

Citrus makes up over half that is 50% of the trash/wastes generated by the juice industries in the form of peel and seed residues (Ozturk *et al.*, 2018), and more than 80 million tons of *C. sinensis* are produced each year (FAOSTAT, 2019).

Citrus sinensis is rich in vitamin C, contains dietary fiber, a potent natural antioxidant, folate (vitamin B-9) which is essential for the production of red blood cells as well as for the growth and function of healthy cells, and other bioactive components like flavonoids and carotenoids that prevent cancer and degenerative diseases (Ejaz *et al.*, 2006). Consuming meals high in vitamin C strengthens the body's defence against infectious diseases and helps the body rid itself of harmful, pro-inflammatory free radicals. Hesperetin and naringenin are among the many phytochemicals found in sweet orange. As an antioxidant, scavenger of free radicals, anti-inflammatory, and immune system modulator, naringenin has a bioactive impact on human health (Etebu & Nwauzoma, 2014).

Maesobotrya Barteri (Bush Cherry)

The flowering plant *Maesobotrya sp* is a member of the Phyllanthaceae family, while some people also classify it as a member of the Euphorbiaceae (Pandey, 2006).

Despite the tree's nutritional and therapeutic value, the specie *M. barteri* is underutilized in Nigeria (Ogbuagu & Agu, 2008). In some parts of Africa, *Maesobotrya species* are used medicinally (Okwu & Ekei, 2003). It produces tasty, tongue-staining, succulent black-purple fruits. The endosperm may or may not be present, and the seeds frequently have a noticeable carbuncle. In the southern part of Nigeria, researchers have examined the nutritional content of *Maesobotrya floribunda's* fruit and seeds (Uduak & Kola, 2010).

Chewing sticks from *Maesobotrya barteri* and other medicinal plants were examined for major, minor, and trace elements by Okwu & Ekei (2003). It was discovered that these chewing sticks, when used without tooth paste, were dependable and highly effective in cleaning the teeth of many people in the southern region of Nigeria. Users of those chewing sticks typically have strong, clean, fresh teeth free of dental plaque. These findings provide the foundation for the samples' ability to prevent and shield teeth from plaques and caries. According to Ntie-Kang *et. al.*, 2013, Plants in the Euphorbiaceae family, including *Alchornea sp., Croton sp., Discoglypremna sp., Drypetes sp., Fontainea sp., Macaranga sp., Maesobotrya sp., Neoboutonia sp., and Uapaca sp* are found to be rich in terpenoids (69.5%).

The brilliant red fruits of *M. barteri* (bush cherry) are excellent and a welcome refreshment for thirsty travellers in Liberia, according to Fauna and Flora International (Emily, 2009). In the Congo, psoriasis is treated with a paste made from crushed *Maesobotrya cordulata* fruits from Central Africa (Kawanabe *et al.*, 1999). According to Ramirez *et al.* (1988), crushed leaves are used to scarification to treat oedema and to wounds to promote healing. Leprosy is treated

in the Congo by drinking and bathing the bark decoction of *Maesobotrya vermeulenii* (Tane *et al.*, 1996).

Therefore, the aim of this research was to extract the oil from *Citrus sinensis* and *Maesobotrya barteri* seeds, determine its physicochemical properties, and determine its antimicrobial activities in order to establish additional uses/applications, such as in the pharmaceutical and cosmeceutical industries, beyond their use in nutrition.

MATERIALS AND METHODS

Sample Collection and preparation

Fresh fruits of *Citrus sinensis* and *Maesobotrya barteri* were bought from the market in Ondo town, Ondo State. Using a knife, the fruits were cut open, and squeezed manually to obtain the seeds from the flesh. The seeds were dried at room temperature for six weeks. After drying, the seeds were de-shelled manually and pulverized with an electric blender, weighed and packed in a container for further analysis.

A glass jar containing the powdered seeds of *Citrus sinensis* and *Maesobotrya barteri* were subjected to a maximum of four days of cold extraction using n-hexane. It was filtered and concentrated using rotary evaporator.

After the oil was air dried to eliminate any leftover solvent, it was weighed. The oil was poured into a sample bottle for further analysis. The percentage yield of both oils was determined

Solvents and Chemical Reagents

All solvents and chemical reagents were obtained from (Pascal Scientific Ltd, Akure) which include: n-hexane, methanol, ethyl acetate, chloroform, ethanol, acetic acid, diethyl ether, potassium hydroxide, potassium iodide, glacial acetic, sodium thiosulphate and hydrochloric acid, etc.

Physicochemical Properties of the oil

The physicochemical characteristics of the oil were determined using the standard methods with a slight modification according to Gerpen (2005); Ibeto *et al.* (2012); Abdulhamid *et al.* (2014); Kayode (2015); Atolani *et al.* (2016);

Acid value Determination

A flask containing precisely 1 g of each oil, 25 ml of diethyl ether, 25 ml of methanol, and 3 drops of phenolphthalein indicator was added to the mixture. The mixture was warmed over a water bath for five minutes before being titrated against 0.1M KOH while being continuously shaken until the pink colour appeared, signifying the end point.

Iodine value Determination

A precise 1 g sample of each oil was weighed into a 250 ml conical flask, and 25 ml of carbon tetrachloride was used to dissolve the oil. The flask was sealed and shaken after 25 ml of wiji's solution was added. One hour was spent letting the mixture stand in the dark. After that, the released/liberated iodine was titrated using a starch indicator against 0.1M sodium thiosulphate. A blank titration was also performed.

Ester value Determination

The difference between the acid value and the saponification value was used to calculate the ester value. Ester value can be determined using the formula below:

Ester value = Saponification value – Acid value

Saponification value Determination

A conical flask was filled with precisely 1 g of each oil, and 25 ml of ethanolic potassium hydroxide was then added. For half an hour (30 minutes), the mixture was refluxed in a bath of boiling water. After adding 3 drops of phenolphthalein, the pink colour was titrated against 0.5 M HCl until it vanished. A blank titration was also performed.

Peroxide value Determination

Each oil sample weighed precisely 0.5 g and was placed in a conical flask with 1 g of potassium iodide. It was mixed with a solvent mixture of 20 ml (13 ml glacial acetic and 7 ml chloroform). The conical flask was placed on a water bath for about one minute after which 20 ml of 5% potassium iodide and 25 ml of water were added to the mixture inside the conical flask. Then, using a starch indicator, this was titrated against a 0.002 M sodium thiosulphate solution until it was colourless Blank titration was also performed.

Specific gravity and Density Determination

A 50 ml measuring cylinder was washed, dried, and weighed was recorded as W_0 in order to establish the specific gravity. 1.4 ml and 1.8 ml of the oil was measured into the measuring cylinder and re-weighed, and was recorded as W_1. The measuring cylinder was washed and dried. 1.4 ml and 1.8 ml of distilled water was measured into the measuring cylinder and weighed, and the value was recorded as W_2. The specific gravity was calculated using the expression;

$$\text{Specific gravity} = \frac{W1-W0}{W2-W0} \quad (1)$$

A 50 ml measuring cylinder was properly washed, dried, weighed, and marked as M_0 in order to calculate density. 1.4 ml and 1.8 ml of the oil was measured into the measuring cylinder and weighed, and was recorded as M_1. The volume of the oil samples was recorded as V_1.

Density was calculated using the expression;

$$\text{Density} = \frac{Mass}{\text{Volume}} \quad (2)$$

Trans-esterification Determination

After weighing a precise 2 g sample of each oil, it was put into a beaker with 10 ml of 0.2 M methanolic HCl. After an hour of refluxing, the mixture was transferred into a separating funnel. After adding 20 ml of n-hexane and 10 ml of water to the separating funnel and shaking it vigorously to allow the two layers to separate, the aqueous layer (water) was decanted into a beaker and then poured back into the separating funnel to be cleaned again with 10 ml of n-hexane. The fatty acid methyl esters (FAMES), which make up the organic layer (oil), were also decanted and kept for GC-MS analysis. The percentage yield of the trans-esterified oil was determined using the expression;

$$\text{Percentage yield} = \frac{weight\ of\ trans-esterified\ oil \times 100}{\text{Weight of lipid}}$$

Antimicrobial Activity of the oils

Agar disk diffusion method was used for bacteria susceptibility testing. 500 ml of distilled water were added to a conical flask containing 28 g of nutrient agar that had been weighed. Additionally, a conical flask was filled with 100 ml of distilled water after 1.3 g of nutrient broth had been weighed into it. A separate conical flask was filled with 400 ml of distilled water, covered with aluminium foil, and autoclaved for 45 minutes to sterilized it.

After cooling to room temperature, the sterilized medium was poured. The agar plate was drilled with a sterile cork borer, and the standardized bacteria and fungi were then dispersed around the plate.

Using a micro pipette or syringe, a 0.5 g sample of each seed oil was added to the hole after being dissolved in 50% DMSO. The clear zone of inhibition's diameter was measured and

recorded in mm after the plates were incubated for 24 hours at 37 ^{0}C (Selvamohan & Sandhya, 2012). The bacteria organisms used are *Streptococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi, Klebsiella pnemonae* and *Pseudomonas aeruginosa and* the fungi organisms used are *Candida albicans, Penicillium notatum, Aspergillus nigar* and *Rhizopus stolonifer*.

RESULTS AND DISCUSSION

Table 1: Percentage yield of oils from seeds of *Citrus sinensis* (Orange) and *Maesobotrya Barteri* (Bush Cherry)

Oil seed	Yield of extract in gram (g)	Percentage yield of extract (%)
C. sinensis	42	36.21
M. barteri	140	12.56

The oil extracted from the seeds of *C. sinensis* (116g) and *M. barteri* (1115g) were weighed and the percentage yield was ascertained.

From the results shown in Table 1; the oil of *C. sinensis* seed has higher percentage yield than that of *M. barteri* seed oil.

Table 2: Physicochemical properties of oils from seeds of *Citrus sinensis* (Orange) and *Maesobotrya Barteri* (Bush Cherry)

Parameter	*Citrus sinensis* (Orange)	*Maesobotrya Barteri* (Bush Cherry)
Acid value (mg KOH/g)	7.55	1.40
Iodine value (gI_2/100g)	83.48	97.05
Ester value (mg KOH/g)	186.03	172.71
Saponification value (mg KOH/g)	193.58	174.11
Peroxide value (meq/kg)	5.72	5.91
Specific gravity (g)	0.86	0.91
Density (g/cm^3)	0.88	0.90
% oil trans-esterified	91.88	88.76
Colour	Golden-yellow	Reddish-Brown
Physical state at ambient temperature (27^0 C)	Liquid	Liquid

The acid value of *Citrus sinensis* seed oil was found to be 7.55 mg KOH/g, while the acid value of *M. barteri* seed oil was found to be 1.40 mg KOH/g which was within the acid value range reported for oil from seed of Palm kernel. The acid value range makes it suitable for soap production (Afolabi, 2008). Also, *M. barteri* seed oil is within the acid value range reported for oil from seed of *Citrillus colocynthis* (melon) according to Omozuwa *et al.* (2024).

Citrus sinensis seed oil had an iodine value of 83.48 gI2/100 g, while *M. barteri* seed oil had an iodine value of 97.05 gI2/100 g. This indicated that *M. barteri* seed oil is slightly richer in unsaturated fatty acid compared to *C. sinensis* seed oil. According to their iodine values, triglyceride oils can be classified as drying, semi-drying, or non-drying. A drying oil's iodine value is greater than 130, whereas a semi-drying oil's is between 90 and 130. If the iodine value is less than 90, the oil is classified as non-drying (Guner *et al.,* 2006), which categorizes *Citrus sinensis* seed oil as non-drying oil which is more suitable for soap production compare to *M. barteri* seed oil as semi-drying oil.

Citrus sinensis seed oil had an ester value of 186.03 mg KOH/g, whereas *M. barteri* seed oil had an ester value of 172.71 mg KOH/g.

Citrus sinensis seed oil had a saponification value of 193.58 mg KOH/g, while *M. barteri* seed oil had a saponification value of 174.11 mg KOH/mg. The high saponification value of both oils represents the presence of lower molecular weight of fatty acids in the oils and therefore falls within the recommended range of edible oils and could also be used in soap making since its falls within the recommended range.

The peroxide value of *Citrus sinensis* seed oil was found to be 5.72 meq/kg, while the peroxide value of *M. barteri* seed oil was found to 5.92 meq/kg. Their low peroxide value, suggested that they could be used for human consumption. According to FAO/WHO (2009), edible oils should have a peroxide value of less than 10 meq O2/kg. Also, low peroxide value serves as indicators of the presence of anti-oxidants in the oils (Bennion, 1995).

Citrus sinensis seed oil had a specific gravity of 0.86 g, while *M. barteri* seed oil had a specific gravity of 0.91 g which is within the specific gravity range reported for *Citrillus colocynthis* (melon) seed oil (Omozuwa *et al.*, 2024), as well as the range of specific gravities reported for a few Nigerian oil seeds (Onyeike & Acheru, 2002). According to Bamgboye and Adejumo (2010) and Britannica, (2023); specific gravity is the ratio of a substance's density to that of water at 4 °C.

Citrus sinensis seed oil had a density of 0.88 g/cm^3, whereas *M. barteri* seed oil had a density of 0.90 g/cm^3 which is within the range of densities reported for oil derived from *Sesamum indicum L.* seeds (Mohammed & Hamza, 2008) and oil derived from African bean seeds (Odoemelam, 2005), suggesting that the oil could be used on a commercial scale.

Citrus sinensis seed oil had a percentage yield of trans-esterified oil of 91.88 % whereas *M. barteri* seed oil gave a percentage yield of trans-esterified oil of 88.76 %.

Citrus sinensis seed oil and *M. barteri* seed oil are both liquid at room temperature with a golden-yellow and reddish-brown colouration respectively. According to Omozuwa *et al.* (2024), *Citrillus colocynthis* (melon) seed oil has a golden-yellow colouration which is the same colour as *Citrus sinensis* seed oil.

Table 3: Antimicrobial Activity of oil from *Citrus sinensis* seeds against pathogens

	Concentrations (mg/ml)						
Pathogens	**200**	**100**	**50**	**25**	**12.5**	**Positive control**	**MIC mg/ml**
	Zone of inhibition (mm)						
Bacteria						Gentamincin (10 mg/ml)	
S. aureus	16±1.0	13±1.0	10±1.0	-	-	36±1.0	50
E. coli	19±1.0	15±1.0	13±0.0	10±0.0	-	35±0.0	25
B. subtilis	17±1.0	14±0.0	12±0.0	10±0.0	-	36±1.0	25
P. aeruginosa	18±0.0	16±0.0	14±0.0	12±0.0	10±0.0	37±1.0	12.5
S. typhi	19±0.0	18±0.0	16±0.0	14±0.0	10±0.0	36±0.0	12.5
K. pnemonae	17±1.0	14±0.0	12±0.0	10±0.0	-	37±1.0	25
Fungi						Tioconazole 70 %	
C. albicans	18±0.0	14±0.0	12±0.0	10±0.0	-	27±1.0	25
A. niger	14±0.0	11±0.0	10±1.0	-	-	27±1.0	50
P. notatum	15±1.0	12±1.0	10±1.0	-	-	26±0.0	50
R. stolonifer	14±0.0	11±1.0	10±0.0	-	-	26±0.0	50

- Means no clear zone of inhibition to measure, values are average of duplicate samples

Key

S. aureus as Staphylococcus aureus, E. *coli* as *Escherichia coli*, *B. subtilis* as *Bacillus subtilis*, *P. aeruginosa* as *Pseudomonas aeruginosa*, *S. typhi* as *Salmonella typhi*, *K. pnemonae* as *Klebsiella pnemonae*, *C. Albicans* as *Candida albicans*, *A. Niger* as *Aspergillus niger*, *P. notatum* as *Penicillium notatum*, *R. stolonifer* as *Rhizopus stolonifer*.

In Table 3, The *C. sinensis* seed oil extract was found very active against most of the tested bacteria even at a very low minimum inhibitory concentration (MIC). For instance, at concentration of 200 mg/ml the oil extract shows high activity against all the tested bacteria organisms especially *E. coli* and *S. typhi* having the highest sensitivity/zone of inhibition at 19 while at concentration of 100 mg/ml the oil extract shows activity against *S. typhi* and *P. aeruginosa* having the highest zone of inhibition of 18 and 16 respectively. Also, at concentration of 50 mg/ml the oil extract shows activity against *S. typhi* and *P. aeruginosa* having the highest zone of inhibition of 16 and 14 respectively whereas at concentration of 25 mg/ml, the oil extract shows moderate activity against *S. typhi* and *P. aeruginosa* having the highest zone of inhibition of 14 and 12 respectively. But at concentration of 12.5 mg/ml, lower activities were recorded against *P. aeruginosa* and *S. typhi* both having the zone of inhibition of 10. This oil had inhibitory activities against *S. typhi* and *P. aeruginosa* at all concentrations tested. However, Activities were lower than the positive control; gentamicin.

The oil extract was found very active against all the tested fungi at concentrations of 200 mg/ml, 100 mg/ml and 50 mg/ml but at concentration of 25 mg/ml, lower activity was recorded against *C. albicans* having the zone of inhibition of 10. Activities were however lower than the positive control; tioconazole.

The minimum inhibitory concentration (MIC) for the antibacterial mostly was 25 mg/ml and 50 mg/ml for anti-fungi respectively.

Table 4: Antimicrobial Activity of oil from *M. barteri* seed against pathogens

	Concentrations (mg/ml)						
Pathogens	**200**	**100**	**50**	**25**	**12.5**	**Positive control**	**MIC mg/ml**
	Zone of inhibition (mm)						
Bacteria						Gentamicin (10 mg/ml)	
S. aureus	17±1.0	16±0.0	14±0.0	12±0.0	10±0.0	35±1.0	12.5
E. coli	17±0.0	14±0.0	12±0.0	10±0.0	-	35±0.0	25
B. subtilis	15±1.0	13±1.0	10±0.0	-	-	35±1.0	50
P. aeruginosa	17±0.0	14±0.0	12±0.0	10±0.0	-	35±1.0	25
S. typhi	15±1.0	13±1.0	10±0.0	-	-	36±0.0	50
K. pnemonae	17±0.0	14±0.0	12±0.0	10±0.0	-	37±1.0	25
Fungi						Tioconazole 70 %	
C. albicans	17±1.0	14±1.0	12±0.0	10±0.0	-	28±1.0	25
A. niger	14±0.0	13±0.0	10±0.0	-	-	27±1.0	50
P. notatum	14±0.0	13±0.0	10±0.0	-	-	27±0.0	50
R. stolonifer	17±0.0	14±0.0	12±0.0	10±0.0	-	26±0.0	25

- Means no clear zone of inhibition to measure, values are average of duplicate samples

In Table 4, The *M. barteri* seed oil extract was found very active against most of the tested bacteria even at a very low minimum inhibitory concentration (MIC). For instance, at concentration of 200 mg/ml the oil extract shows high activity against all the tested bacteria organisms especially *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pnemonae*, all having the highest sensitivity/zone of inhibition at 17 while at concentration of 100 mg/ml the oil extract shows activity against *S. aureus*, *P. aeruginosa* and *K. pnemonae* having the highest zone of inhibition of 16, 14 and 14 respectively. Also, at concentration of 50 mg/ml the oil extract shows activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pnemonae* having the highest

zone of inhibition of 14, 12, 12 and 12 respectively whereas at concentration of 25 mg/ml, the oil extract shows moderate activity against *S. aureus* with a zone of inhibition of 12 while *E. coli*, *P. aeruginosa* and *K. pnemonae*, all having the zone of inhibition of 10. But at concentration of 12.5 mg/ml, lower activity was recorded against *S. aureus* having the zone of inhibition of 10. This oil had inhibitory activity against *S. aureus* at all concentrations tested. However, Activities were lower than the positive control; gentamicin. The oil extract was found very active against all the tested fungi at concentrations of 200 mg/ml and 100 mg/ml whereas at concentration of 50 mg/ml, the oil extract was found active against *C. albicans* and *R. stolonifer* having the highest zone of inhibition of 12 but at concentration of 25 mg/ml, lower activities were recorded against *C. albicans* and *R. stolonifer* both having the zone of inhibition of 10. Activities were however lower than the positive control; tioconazole. The minimum inhibitory concentration (MIC) for the antibacterial mostly was 25 mg/ml whereas the MIC for anti-fungi was 25 mg/ml and 50 mg/ml.

CONCLUSION

The results from this study showed that oil from seeds of *Citrus sinensis* and *M. barteri* have great potential because they may be used productively in homes as edible oils, as well as serving as raw materials for the pharmaceutical and cosmetic industries. But oil from seed of *Citrus sinensis* is more suitable for the production of soap and cream because of its non-drying oil properties production compare to *M. barteri* seed oil. Also from the results, oil from the seeds of *Citrus sinensis* and *M. barteri* show appreciable antimicrobial activity against all the tested organisms (bacteria and fungi). However, oil from seed of *Citrus sinensis* demonstrated remarkable and excellent antimicrobial activity than the oil from seed of *M. barteri* against *E. coli*, *S. typhi* and *C. albicans* at concentration of 200 mg/ml. Utilization of these seeds for residential and industrial usage in cosmetics and pharmaceutical will reduce environmental wastes and enrich their commercial value. Additionally, they offer legitimate channels for the pharmaceutical and cosmetic industries to channel these underutilized bio-resources (seeds) into medicines, soaps, and creams.

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