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Original Research Article

Phytochemical, Antimicrobial and GC-MS Analyses of Extracts of *Annona Muricata* l. (Sour SOP) Leaf

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ABSTRACT: The plant, *Annona muricata* is commonly known as sour-sop and both the leaves and fruits are used globally to manage various forms of infections. The leaf of *Annona muricata* was investigated to confirm its antimicrobial properties and to identify some of its chemical constituents. The air-dried, powdered leaf obtained from Bwari Area Council, Abuja, was extracted with ethanol to obtain the crude extract. The crude ethanol extract was subjected to phytochemical screening which showed the presence of tannins, quinones, phenols, terpenoids and saponins. The crude extract was also screened for antimicrobial activity against some human pathogens, including *Staphylococcus aureus*, *Salmonella typhi* and *Eschericia coli*. The crude extract showed activity against the test organisms at MIC values of 12.5 mg/mL, 12.5 mg/mL, and 25mg/mL, respectively. The fresh leaf was steam distilled and the volatile oil was subjected to GC-MS and FTIR analyses which revealed the presence of 16 major components, mainly alcohols, acids, esters, and oxygenated derivatives of long-chain hydrocarbons. Among them ethanol, 2-2 (ethoxyethoxy)- was the major component constituting about 89% of the total volatile oil. Of great significance was the presence of eugenol, an aromatic *monoterpenoid*. The presence of these chemical components in the leaf extracts of *Annona muricata* may account for its ethno-medicinal uses in the management of infections.

Keywords: *Annona muricata* (Sour sop) leaves, phytochemicals; antimicrobial activity; GC-MS analysis, steam-volatile oil

INTRODUCTION

Extracts of various parts of higher plants as well as their essential oils are reservoirs of biologically active substances and are of benefit to man and animals by protecting them against pathogens. Essential oils are also used as food preservatives, and as flavouring, antimicrobial, analgesic, sedative, anti-inflammatory and local anesthetic agents (Florence and Jeeva, 2016).*Annona muricata* belongs to the plant domain Eukaryota, kingdom Plantae, phylum Spermatophyta, sub-phylum Angiospermae, class Dicotyledonae, order Annonales, family Annonaceae and genus *Annona. A. muricata* L. has different names in different communities; Abo, Chop-chop, Sawansop, Sapisapi (Nigeria), soursop (English), graviola (Portuguese), guanábana (Latin American and Spanish), sinini (Bolivia) (Ana and Eva, 2018). .*A. muricata* is local to the hottest tropical regions in South and North America, presently is broadly circulated all through tropical and subtropical areas of the world, including India, Malaysia, Australia and Africa (Sejal and Jayvadan, 2016). *A. muricata* is an evergreen, earthbound, erect tree reaching 5–8 m in stature and provides an open, roundish covering with enormous reflexive, dull green leaves. According to some reports every part of *A. muricata* tree is broadly utilized in

traditional medicine against a variety of human afflictions and illnesses, particularly cancer and parasitic diseases (Yahaya, *et al,* 2017; Moghadamtousi, *et al.*, 2015, Sejal and Jayvadan, 2016). Previous phytochemical investigations have shown the presence of alkaloids, phenolics, megastigmanes and acetogenins which were reported to constitute the major chemical constituents of the Annonaceae family (Yang et al., 2015; Coria-Tellez et al., 2018). Acetogenins are a unique group of derivatives of long chain fatty acids derived from the polyketide pathway (Gavamukulya et al., 2017). In this work we report the antimicrobial activity of the crude ethanol extract against 3 common human pathogens and the identification of 16 components including eugenol, an aromatic monoterpenoid, and a number of aliphatic components with ethanol,2-(2-ethoxyethoxy)- as the major constituent(89.06%), using GC-MS analysis.

MATERIALS AND METHODS

Materials

The fresh leaves were obtained from Bwari Area Council, Abuja, Nigeria, authenticated at the Herbarium Unit of the Department of Biological Sciences, Veritas University Abuja, Nigeria. They were washed and dried under a cool atmosphere and powdered before extraction. The solvents used in the study were of standard grade and were re-distilled before use. Silica gel for TLC was mesh size 70-230 coated on aluminium foil. The TLC spots were viewed under UV lamp (254nm and 366nm).The organisms for antimicrobial tests included *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.* The test organisms were clinical isolates obtained from the Department of Microbiology, Veritas University and were maintained on nutrient agar slants. Aliquots of nutrient broth (20ml) were inoculated with the culture of test organisms using a loop and then incubated at 37°C for 24 h.

Gentamicin was used as the reference antibiotic (positive control) while water was used as the blank (negative control). IR spectrum was obtained on Model JPG Cary 630 by Agilent Technologies. Gas-Chromatography/Mass Spectrometry (GC-MS) analysis was performed using Agilent 190915-433UI equipped with flame Ionization detector (FID). The injection was conducted in splitless mode at 250ºC for 3min at an injection volume of 1µL, average velocity of 35.97 cm/sec, pressure of 7.3614 psi and a flow rate of 0.97414 mL/minute. Chromatographic separations were performed by using analytical column 30 m x 0.25 mm with helium as carrier gas at a constant flow rate of 1.58ml/min. The oven temperature was programmed at 80ºC for 2 min, followed by an increase (held for 4 min), and finally at 250 ^{0}C (held for 5 min). The temperature of the FID was set to 250ºC. Operating conditions (electron impact ionization mode) were an ion source temperature of 200ºC, ionization voltage of 70 eV and mass scan range of m/z 40-500.

Methods

Extraction of plant material

Steam distillation was performed on the fresh leaves (77.8 g). The aqueous distillate was extracted with diethyl ether in a separating funnel, and the ether was evaporated to dryness to yield the volatile oil (0.03g). Maceration was used to extract the dry powdered leaf (186g) with ethanol (2,890 ml). The crude extract (14.7 g) was filtered and evaporated to dryness in a Rotary evaporator at 40°C, yielding a dark-green residue. TLC of the crude extract was performed on an aluminum plate with pre-coated silica gel and solvent combinations of hexane and ethyl acetate, and the visible spots were counted to determine the quantity of isolable main components. Following the extraction, the various extracts were collected and concentrated using a rotary evaporator at reduced pressure. The extract was concentrated to a concentration of 55°C.

Preliminary phytochemical analysis

The crude extract was analyzed for phytochemicals such as sterols, terpenoids, phenols, quinones, saponins, reducing sugars, flavonoids and tannins using the procedure described by Okah and Okwute (2020)

IR and GC-MS analysis of volatile oil from steam-distillate

The volatile oil from fresh leaves steam-distillate was subjected to GC-MS analysis. The identification of the peaks was computer generated by comparing their mass spectra with those of the bibliography data of known compounds from the NIST library mass spectra database while the % Areas were estimated according to the method of Wanakhachornkrai and Lertsiri, (2003).

Antimicrobial screening

Antimicrobial activity of the crude extract was determined by well-diffusion method on Mueller Hinton agar medium. The media were prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes. It was screened against the following micro-organisms; *Escherichia coli (Ec), Staphylococcus aureus (Sa)* and *Salmonella typhi (St)* using a standard procedure (Okwute and Nwosu, 2022). Different dilutions of the extracts were prepared to give

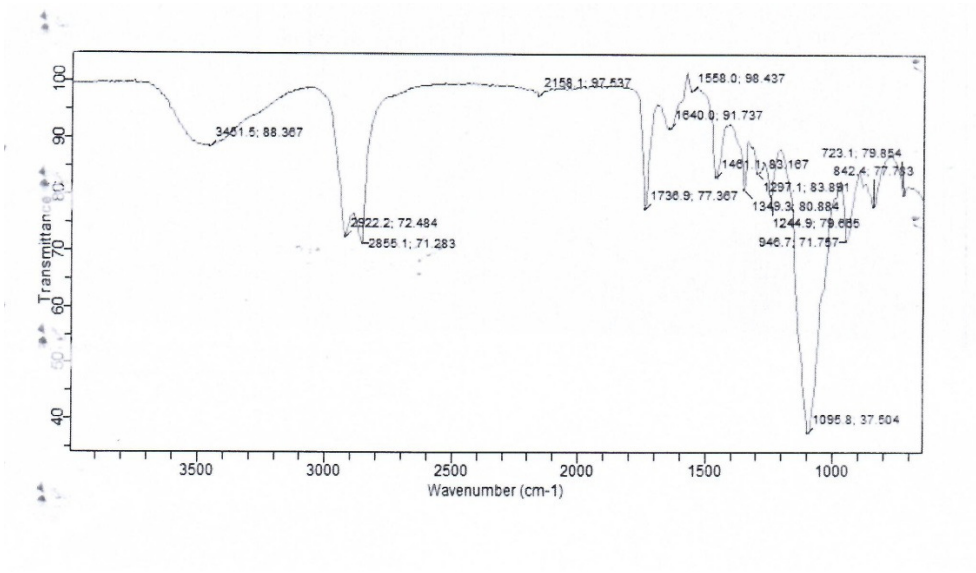


Figure 1: IR Spectrum of volatile oil of *Annona muricata* leaf

Table 1: GC-MS Analysis Data on Steam-distillate of fresh Leaves of *Annona muricata.*

Peak No	RT(Mins.)	Area%	Name of Component	% Quality
1.	5.808	0.35	1-Propanol,2-methyl-	64
2.	6.542	0.67	Acetic acid	83
3.	7.881	0.33	Ethanol,2-ethoxy-	80
4.	11.760	0.21	Propylene glycol	43
5.	14.970	0.59	O-Methylisourea	09
6.	18.847	89.06	Ethanol,2-(2-ethoxyethoxy)-	64
7.	20.334	0.89	Ethanol,2,2'-oxybis-	78
8.	21.625	3.10	Methyl 2-(2-(2-ethoxyethoxy)ethoxy acetate	64
9.	23.870	1.96	2-(2-Hydroxyethoxy) ethyl acetate	72
10.	28.648	0.33	2,4-Decadienal	76
11.	29..066	0.21	Bromoacetic acid, tetrdecyl ester	38
12.	29.737	0.83	Eugenol	94
13.	32.150	0.23	Propyl n-butyl disulfide	12
14.	32.929	0.20	Hexaethylene glycol	53
15.	34.436	0.18	Ethane,1,2-diethoxy-	50
16.	35.117	0.22	Pentadecanoic acid, 14-methyl-, methyl ester	90

final concentrations of 100.0, 50.0, 25.0 and 12.5 mg/mL^{-1}. A standard cork borer of 9 mm diameter was used to bore a well and 2mls of each concentration of the ethanolic extract was transferred into the well with a needle syringe. Gentamicin was taken as positive control while water as negative control. The plates were incubated at 37ºC for 24 hrs. Then antibacterial activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

In view of the fact that the fresh leaves of sour sop plant are usually taken as tea, the cleaned fresh leaves were steam-distilled and the volatile oil from the hydro-distillate was subjected to FTIR analysis to assess the various functional groups present and therefore determine the classes of natural products which constitute the volatile chemical components. The IR spectrum (Figure 1) of the volatile oil showed aromatic ring C=C skeleton, carbonyl (C=O), hydrocarbon skeleton(C-H) and hydroxyl (OH) absorptions at 1640, 1736.9, 2855.1, 2922.2 and 3451.6, respectively. This suggests the presence of aromatic nucleus, aliphatic skeletons, carbonyl compounds (esters, acids, aldehydes) and alcohols in the mixture. It was also subjected to GC-MS analysis to identify the volatile components. The gas chromatogram and the volatile components are presented in (Figure 2 and Tables 1, 2), respectively. The analysis gave 16 different

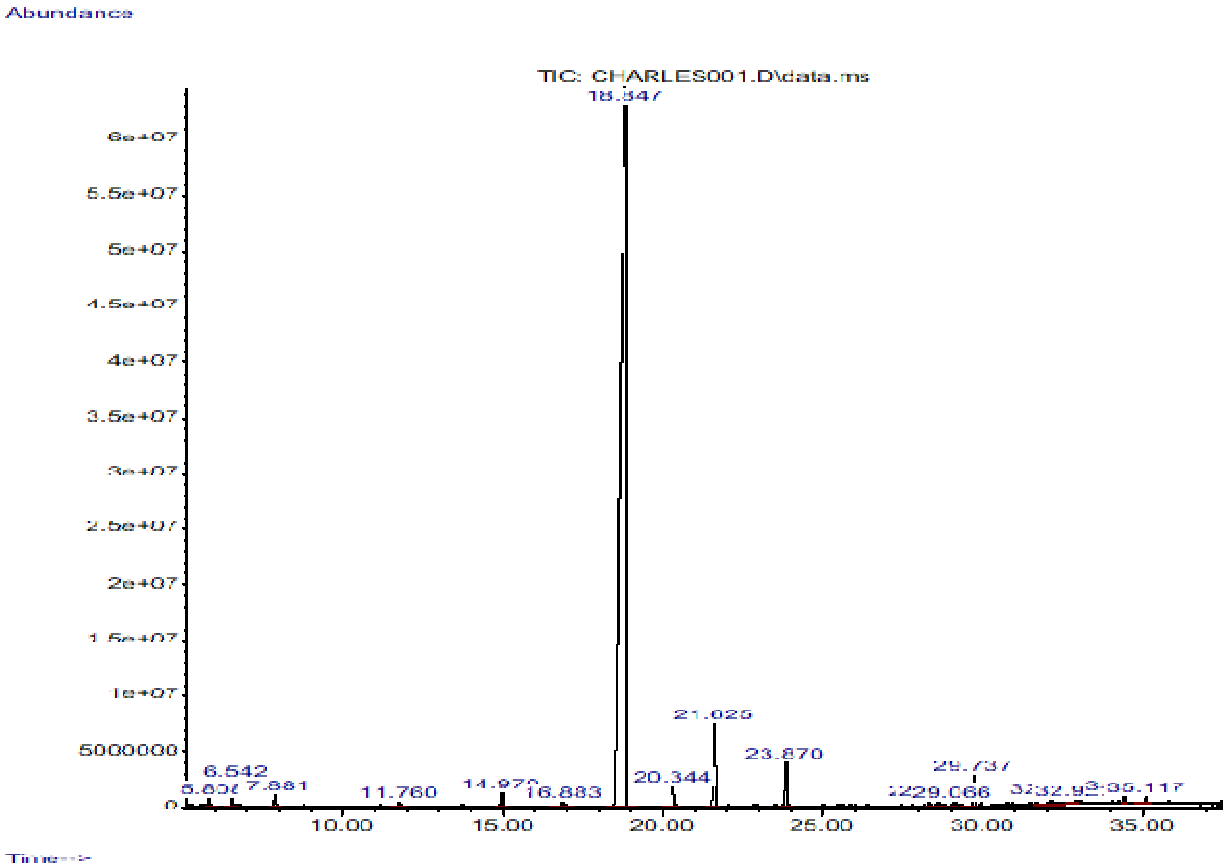


Figure 2: Gas chromatogram of volatile oil of fresh leaf of *Annona muricata*

Table 2: Phytochemical screening of crude ethanol extract of *Annona muricata* leaf.

Phytochemicals	Remarks
Sterols	-
Terpenoids	+
Quinones	+
Flavonoids	-
Phenols	+
Saponins	+
Reduced sugars	-
Tannins	+

Key: (+)=Present; (-)=Absent

Table 3: Antimicrobial screening of the ethanol extract of *Annona muricata* leaf.

Microorganisms	Zone of Inhibition(mm)/Concentration(mg/ml)					
	100	50	25	12.5	GT(100mg)	Water
Staphyloccocus aureus	21	16	10	8	25	0
Escherichia coli	21	12	10	8	25	0
Salmonella typhi	18	10	8	4	25	0

Key: GT=Gentamicin (+ve Control); Water=Control (-ve)(Water without extract or antibiotic)

components (Table 3)**,** mainly oxygenated derivatives of long-chain hydrocarbons. Ethanol, 2-2 (ethoxyethoxy)-,**1** was the major component constituting about 89% of the total volatile oil. Though, the molecular mass is expected to appear at m/z 134(M^+), the fragment ion peak at m/z 116 (M^+-18) by loss of H_2O is more stable and is observed

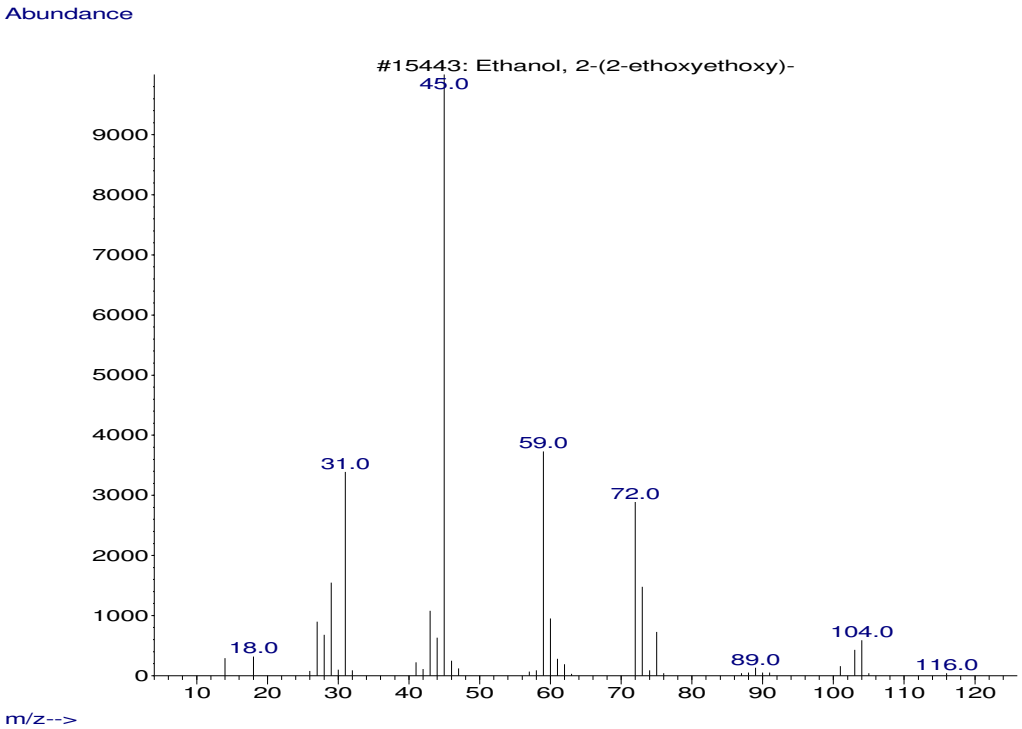
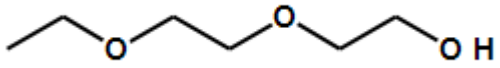
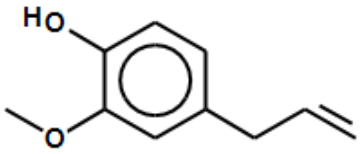


Figure 3: MS of ethanol, 2-2(ethoxyethoxy)- from volatile oil of *A.muricata* leaf

in the MS (Figure 3). Of significance is the presence of eugenol (0.83%), **2,** an aromatic monoterpenoid (C_{10}) in the steam-distillate. This oil which has traditionally been extracted from essential oils from clove, cinnamon, basil and bay leaf is used as a flavouring agent for foods and teas and as an herbal oil to treat toothache as well as gastrointestinal and respiratory problems (Jie *et al*., 2021).





The dry powdered leaf was extracted with ethanol and the crude extract was subjected to preliminary phytochemical analysis. The phytochemicals found in the leaf are recorded in (Table 2). The results are in agreement with the identification of eugenol, a monoterpenoid and a phenol, in the volatile oil of the leaf. Also, no sterols and flavonoids were found in the volatile oil and the crude extract. Saponins have considerable potential as pharmaceutical and nutraceuticals agents in natural or synthetic form. Saponins, from a variety of sources have been shown to have hypocholesterolemic, anti-coagulant, anti-carcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory and antioxidant activity (Rao and Gurfinkel, 2000). Tannins, including gallo and ellargic acid (epigalli-tannins), are inhibitors of HIV replication. For example, punicatin, punicalagin are known to inhibit HIV replication in infected H9 lymphocytes with little cytotoxicity (Nonaka *et al.,* 1990). Hydrolysable tannins have also shown potential antibacterial effects against *Helicobacter pylori* (Funatogawa *et al.,* 2004). Phenolics have also been shown to be potent antibacterial and antiviral agents. For instance, phenolics constrained the growth and proliferation of hepatitis C virus (HCV); this virus is a primary blood-borne pathogen causing liver cirrhosis and hepatocellular carcinoma (HCC), thus inhibiting infection in primary human hepatocytes (Hsu *et al.,* 2015). The anti-carcinogenic capacity is a primary disease-preventive effect of phenolics; they retard the initiation and progression of cancers by constraining the transformation of normal cells, the growing tumours, angiogenesis, and metastasis. The anti-carcinogenic capacity is a primary disease-preventive effect of phenolics; they retard the initiation and progression of cancers by constraining the transformation of normal cells, the growing tumours, angiogenesis, and metastasis

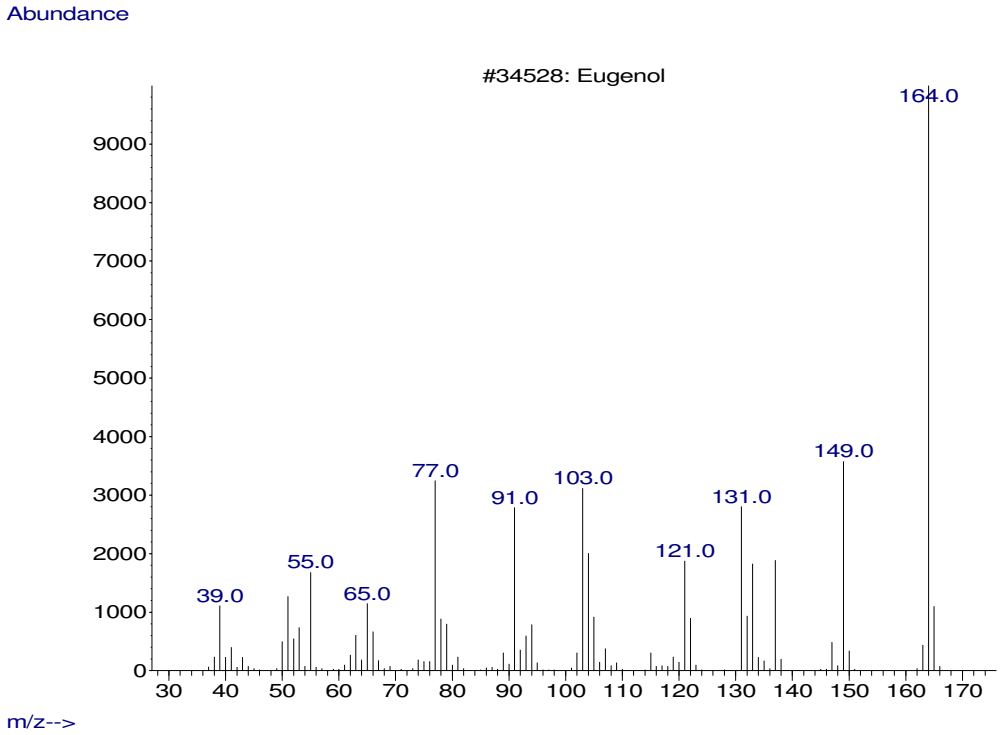


Figure 4 : MS of eugenol from volatile oil of *A. muricata* leaf

(Anantharaju *et al.,* 2016). The crude ethanol extract was also subjected to antimicrobial screening against 3 human pathogens of economic significance. The results are shown in (Table 3). From Table 3, the crude extract showed reasonable activity against the human pathogens, *Staphylococcus aureus, Escherica coli* and *Salmonella typhi* when compared to the standard antibiotic, gentamicin, at a concentration of 100mg/ml. Thus, supporting its uses in ethno- medicine.

Conclusion

This work has shown that the volatile components of the fresh leaf steam-distillate of *Annona muricata* are alcohols, fatty acid derivatives and eugenol that have a wide range of therapeutic applications. In the work it is significant that an aromatic monoterpenoid, eugenol, was identified as a component of the volatile oil of the leaves. These components along with those identified through phytochemical analysis may account for the bioactivity of crude leaf extract against some human pathogens tested in this work, and therefore explains the ethno-medicinal uses of the plant for the management of infections.

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