

# Phytochemical Study and Evaluation of the Antimicrobial Activity of *Terminalia avicennioides* (Guill and Perr) Leaf Extracts on the Bacteria Involved in Common Infections in Niger

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## ABSTRACT

**Background and Objective:** The leaves of *Terminalia avicennioides* are used in Western Niger against gastric disorders and skin infections. The objective of the study is to perform phytochemical screening of the leaves of *Terminalia avicennioides* extracts and test their antimicrobial activity with special interest in the bacteria most involved in diarrheal infections *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi* and *Staphylococcus aureus* and reference. **Materials and Methods:** The methanol and aqueous extracts of this plant were prepared with the leaf powder. Aqueous extract was prepared by decoction and the methanolic extract was prepared by maceration. The major families of secondary metabolites were searched in the crude extracts following standard characterization methods. The extract was evaluated for the presence of alkaloids, flavonoids, tannins, saponins, steroids and glycosides. The evaluation of the antibacterial activity of the extracts was carried out using the disk diffusion method. The MICs and MBCs were used to investigate the antimicrobial activity. **Results:** Phytochemical research revealed the presence of phenols, tannins, flavonoids and steroids. Leaves extract showed high levels of phenolic compounds which are derivatives of gallic acid and a significant level of flavonoid and tannins: Moderate antibacterial activity against clinical strains *Salmonella typhi*, *Staphylococcus aureus* and *Shigella flexneri* and showed no effects on *Escherichia coli*. Better antimicrobial activity was observed with an ethyl acetate fraction of the methanolic extract. **Conclusion:** Phytochemical studies on *Terminalia avicennioides*'s leaves revealed a variety of phenolic compounds which have many biological activities. Thus, it can explain the traditional basis for using this plant in the treatment of various diseases such as anti-inflammatory, hemostatic, cicatrizing and antidiarrheal.

## KEYWORDS

Medicinal plant, gastroenteritis, antibacterial activity, polyphenol, *Terminalia avicennioides*, diarrheal diseases

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## INTRODUCTION

Diarrheal diseases constitute a serious public health problem worldwide. It is the second leading cause of global mortality among children under the age of 5 years<sup>1</sup>. In Niger, 12% of child deaths are caused by diarrheal diseases. The bacteria most involved in diarrheal infections are *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi* and *Staphylococcus aureus*<sup>2</sup>. Several plants are used in traditional medicine in the treatment of non-specific gastroenteritis with satisfactory results. *Terminalia avicennioides* Guill and Perr (Combretaceae) is one of the West African savannah plants whose barks and roots are commonly used in the treatment of gastric disorders, haemorrhoids, stomach ulcers and skin infections<sup>3</sup>. The root barks of the plant have been used in the treatment of various types of diseases such as diarrhoea<sup>4</sup>, malaria<sup>5</sup> and trypanosomes<sup>6</sup>.

The ethanolic extract of the root bark showed moderate antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus*, *Microsporum audouinii*, *Penicillium* species and *Trichophyton rubrum*<sup>7</sup> and significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*<sup>8</sup>. The MIC of root crude extract on *Bacillus subtilis*, was 0.1 and 10 µg/mL for *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. It was not active on *Salmonella typhi*. The crude methanolic stem bark extract showed activity on only *Escherichia coli* with MIC of 10 µg/mL<sup>9</sup>.

Phytochemical screenings carried out on aqueous and ethanol crude extracts from different parts of *T. avicennioides* show the presence of phenolic and terpenes compounds in the plant. Carbohydrates, cardiac glycosides, saponins, flavonoides, tannins, alkaloids, phenols and triterpenes were detected in all the aqueous and ethanol crude extracts of leaf, stem and root barks of the plant<sup>10</sup>.

Furthermore, phytochemical screening of the methanolic, extract carried out by Mann *et al.*<sup>8</sup> revealed the presence of glycosides, saponins, tannins, alkaloids, steroids, phenol and ellagic acid in the root barks extract, but no detect flavonoid. Mann *et al.*<sup>8</sup> found that the absence of alkaloids, steroids, saponins and flavonoids in the leaf extract.

Phenolic acids, flavonoids, triterpenes and triterpenoidal glycosides are also present in high amounts in various *Terminalia* species<sup>11</sup>, Hydrolysable tannin compounds including ellagic acid, punicalagin, flavogallonic acid and terchebulin were isolated from *T. avicennioides* extracts<sup>12</sup>. In another study, a bioguided fractionation of root bark extract with petroleum ether isolated a corresponding triterpene from Friedelin.

Friedelin's antimycobacterial activity was evaluated against Bacillus Calmette Guérin (BCG) with a MIC value of 4.9 µg/mL<sup>13</sup>. Friedelin had previously exhibit antifeedant and anti-inflammatory activities, without cytotoxicity and significant hepatoprotective activity<sup>14</sup>.

The present work aims to carry out a phytochemical screening of extracts from the leaves of *Terminalia avicennioides* and to evaluate their antimicrobial activity on clinical bacterial strains of the enterobacteriaceae genus: *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi* and *Staphylococcus aureus* and their reference strains

## MATERIALS AND METHODS

**Study area:** The leaves of *T. avicennioides* were collected from Kollo, East of Niamey about 28 km, in September, 2022. The extractions, chemical screening and dosage of phenolic compounds were carried out in laboratory of Natural Substances Faculty of Science and Technology, Abdou Momouni University (last trimester 2022). Antibacterial activity on clinical strains was evaluated in the Laboratory of Bacteriology and Mycology, Amirou Boubacar Diallo National Hospital (CHU-Niamey) (2023). The study of the antibacterial activity on the reference strains was carried out in the National Laboratory of Public

Health and Expertise (LANSPEX- Niamey) (2023). The analysis of phenolic compounds was carried out at ICAR-USAMV-University of Agricultural Sciences and Veterinary Medicine (USAMV) Cluj-Napoca.

**Plant material:** The leaves of *T. avicennioides* were collected in September, 2022, the end of the rainy season, from Kollo about twenty kilometers east of Niamey and then identified in the Biology Department of the Faculty of Science, Abdou Moumouni University of Niamey. The leaves of this plant were well-dried and then ground to powder.

**Biological material:** The microbial support consists of clinical bacterial strains: *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi* and *Staphylococcus aureus* isolated from biological fluids in the Bacteriology and Mycology Laboratory of Niamey University Hospital. The reference bacterial strains used are *Shigella flexneri* (S2005BAEEQ), *Salmonella typhi* (S8250H1), *Escherichia coli* (E20081AEEQ) and *Staphylococcus aureus* (SATCC 29213). These strains were provided by the medical and health research center (CERMES) of Niger.

**Preparation of plant extracts:** Powder from the leaves of *T. avicennioides* is used to prepare aqueous and alcoholic extracts.

The preparation of the aqueous extract was made by decoction: About 100 g of the vegetable powder was placed in a 2 L flask and added 1000 mL of water. The mixture is brought to reflux for 30 min. After cooling, the decoction is filtered through hydrophilic cotton. The filtrate is dried to yield the aqueous extract (EAT).

**Preparation of the methanolic extract of *Terminalia avicennioides*:** The alcoholic extract was prepared by maceration in a hydroalcoholic solution. In 1000 mL of 70% methanol, 100 g of vegetable powder is added. The mixture is left stirring, at room temperature for 24 hrs. The fluids were then filtered using a Whatman No.1 filter and remove the solvent using a rotary evaporator (Evaporator Rotate BUCHI R-300). The methanolic extract (EMT) is thus obtained.

**Fractionation of the methanolic extract:** Fractionation of the crude methanolic EMT extract was done by liquid-liquid extraction using solvents of increasing polarity.

In a 50 mL Erlenmeyer flask, 1 g of EMT is dissolved in 20 mL of methanol. The alcoholic solution thus obtained undergoes liquid-liquid extraction with a succession of organic solvents in order of increasing polarity (hexane, dichloromethane and ethyl acetate). The extracts from each solvent are combined and the solvent is evaporated to give the corresponding fraction. Thus EMTF1 corresponds to the hexane fraction, the dichloromethane fraction is EMTF2 and EMTF3 is the acetate fraction. The remaining fraction in methanol is EMTF4.

**Phytochemical screening:** A primary screening consisting of determining the presence of significant secondary metabolite groups was carried out on the crude extracts of *T. avicennioides* leaves. The classic characterization methods of Bruneton<sup>15</sup> were used to detect phenolic compounds by FeCl<sub>3</sub> test, flavonoids by cyaniding test, terpenes by Liebermann-Burchard test and alkaloids by Mayer and Dragendorff's tests.

**Determination of total phenolic compounds:** The total polyphenol content of the methanol extract of *Terminalia avicennioides* leaves was determined using the Folin-Ciocalteu method<sup>16</sup>. As 2 mL of the extract, 10 mL of Folin-Ciocalteu reagent diluted 1/10 and 8 mL of sodium carbonate (20%). The solution obtained is heated to 50°C for 15 min. The absorbance is read using a UV-visible spectrophotometer (SHIMADZU UV-1800, Japan) at a wavelength of 765 nm. Gallic acid is used to establish a calibration curve. The total polyphenol content is expressed in mg of gallic acid equivalent per gram of extract (mg EAG/g of extract).

**Determination of total flavonoid compounds:** The determination of flavonoids was carried out by a method based on the formation of a complex between aluminum chloride and flavonoids. The protocol used is based on that described by Zhishen *et al.*<sup>17</sup> and Kim *et al.*<sup>18</sup>, with some modifications.

A solution of 0.75 mL of 5% sodium nitrite and a solution of 0.75 mL of 10% aluminum chloride was added to 2.5 mL of extract solution. Leave to stir for 5 min then add 5 mL of sodium hydroxide solution (1 N) to the mixture. After 30 min of incubation in the dark, the absorbance of the final solution at 510 nm is recorded. Another solution without plant extract is taken as a reference. The quantity of flavonoids is obtained using a calibration curve carried out with quercetin.

**Determination of condensed tannin:** The condensed tannin content was determined by the vanillin method described by Julkunen-Titto<sup>19</sup>. As 50 mL of the extract, 1.5 mL of the 4% vanillin solution in methanol is added. The mixture is vigorously stirred for 5 min and 0.75 mL of concentrated hydrochloric acid is added. The mixture obtained is left to stand for 20 min. The absorbance was measured at a wavelength of 550 nm. The content of condensed tannins is expressed in milligram catechin equivalent per gram of extract (mg EAT/g of extract) by referring to the catechin calibration.

**Identification of phenolic compounds:** The methanolic extract, richer in polyphenols, was analysed by HPLC chromatography system equipped with a UV-visible tuneable detector at the variable wavelength (190-400 nm). The analyses were carried out at two different wavelengths, 280 and 320 nm.

The analysis of phenolic compounds was carried out using an HP-1200 liquid chromatograph equipped with a quaternary pump, an autosampler, a DAD detector and an API-electrospray MS mono quadrupole detector, -6110 (Agilent-Technology, United States). The column is a Kinetex XB-C18 (5 µm; 4.5×150 mm i.e.) from Phenomenex, USA.

The mobile phase was (A) water acidified with 0.1% acetic acid and (B) acetonitrile acidified with 0.1% acetic acid. The following multistep linear gradient was applied: Start with 5% B for 2 min; from 5 to 90% B in 20 min, maintain for 4 min at 90% B, then 6 min to reach 5% B. The total analysis time was 30 min, flow rate 0.5 mL/min and oven temperature 25±0.5°C.

**Chemical products:** Acetonitrile, HPLC gradient Merck (Germany) and water were purified with a Millipore Direct-Q UV system (USA). The pure standard of gallic acid, chlorogenic acid (99% HPLC purity) and rutin (99% HPLC purity) was purchased from Sigma (USA).

**Determination of antibacterial activity of crude extracts and fractions:** The antimicrobial activities were examined by the disk-diffusion method<sup>20</sup>. The bacterial suspension is made from bacterial culture and physiological water to achieve a turbidity equivalent to the 0.5 McFarland standard. Colonies of the same morphology were taken to avoid the selection of an atypical variant. Discs of Whatman No. 1 paper were cut, sterilized and impregnated with plant extract from a concentration of 100 mg/mL. Each disk was impregnated with 15 µL of plant extract. Petri dishes containing Mueller-Hinton agar were inoculated by swabbing with the previously prepared bacterial inoculum. As 10 min later, the discs were gently placed on the surface of the inoculated agar. Antibiotic reference disks (Ar) are used as a positive control and, depending on the case, disks impregnated with distilled water, methanol, or ethyl acetate constituting the negative control (CN), were also placed on the medium depending on the sample used. After 30 min of incubation at room temperature for pre-diffusion, the boxes were incubated at 37°C in the oven for 24 hrs. Antimicrobial activity was estimated by measuring the inhibition diameter around the discs. The assessment of the effectiveness of the extracts was made according to the Ponce *et al.*<sup>21</sup> criterion.

**Minimal inhibitory concentration (MIC):** The MIC of extracts against the clinical and reference bacteria was evaluated using broth dilution method. The broth dilution method was used to determine the minimum inhibitory concentration of the leaf extract of *T. avicennioides* against different bacterial strains. Increasing concentrations of geometric ratio 2 of the extract were introduced into a series of test tubes (3.12, 6.25, 12.5, 25.0, 50.0 mg/L). In each tube, 1.0 mL of extract and 9.0 mL of Mueller-Hinton culture medium were placed. A quantity of 10.0 mL of bacterial culture broth in the same medium is then added to each tube.

A double series of tubes is realized and incubated at 37°C for 24 hrs. A solution of extract in the Mueller-Hinton broth medium is taken as the negative control and the microbial suspension in the same medium is the negative control. The turbidity of the contents of the tubes indicated the bacterial growth degree. The lowest concentration that inhibits bacterial growth is the minimum inhibitory concentration (MIC). In some tubes containing higher extract concentrations, no growth is observed. While positive control comprised Mueller Hinton broth with test bacteria

**Minima bactericidal concentration (MBC):** The tubes with a concentration higher than the MIC, therefore having shown no visible microbial growth, are each transplanted onto Mueller-Hinton agar. Incubation is carried out again at 37°C for 24 hrs. The lowest concentration for that no microbial colonies are observed is the minimum bactericidal concentration (CMB).

## RESULTS

After the extraction process, the aqueous crude extracts of the leaf of *T. avicennioides* showed yields of 18.1%. While the yields from methanol crude extracts were 16.7%.

The phytochemical screening carried out on the leaves using qualitative methods revealed the presence of tannins, saponins, flavonoids, phenols and steroids in the aqueous and methanol crude extracts. The results of the determination of phenolic compounds concentration in the crude extracts are grouped in Table 1. The quantitative analysis of total phenols, flavonoids and tannins shows that the methanolic extract is richer in phenolic compounds than the aqueous extracts.

The spectrochemical analysis shows that all the phenolic compounds are derivatives of gallic acid (Fig. 1 and Table 2).

The results of the susceptibility test of clinical isolates of *E. coli*, *S. flexneri*, *S. typhi* and *S. aureus* and references showed that the aqueous and methanol extracts of the leaf exhibited inhibitory activities

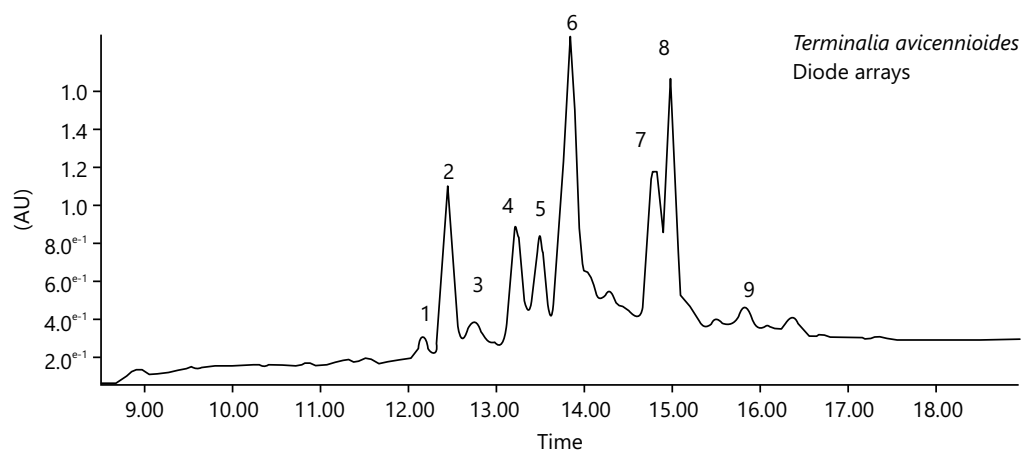


Fig. 1: Chromatograms of the methanolic extract of *Terminalia avicennioides* leaves

Table 1: Phenolic compound contents (mg/g of dry extract) of aqueous and methanolic extracts of *Terminalia avicennioides*

Contents of dry extract (mg/g)	Aqueous extract	Methanolic extract
Polyphenols (mg gallic acid equivalent/g extract) EAG	236.15±1.11	304.95±24.15
Flavonoids (mg quercetin equivalent) EQ	11.00±0.9	15.75±3.55
Condensed tannins (mg catechin equivalent) EC	11.50±0.41	13.70±0.12

Table 2: UV-Visible absorption of phenolic and methanolic compounds from *Terminalia avicennioides* extract

Pic	TR	$\lambda_{1max}$	$\lambda_{2max}$	Proposed compound
1	12.16	214	273	Ethyl gallate
2	12.41	218	268	Methyl gallate
3	12.75	215	275	Gallic acid
4	13.26	217	276	Galloylglucose
5	13.53	217	278	Gallotannin
6	13.85	222	275	Galloquinic
7	14.84	219	278	Ellagitannin
8	15.01	254	365	Ellagic acid
9	16.38	216	277	Digalloyl glucose

Table 3: Diameter of the inhibition zones of the crude aqueous and methanolic extracts and fractions

Bacteria	Ar/ $\phi$	EAT	EMT	EMTF2	EMTF3	EMTF4
<i>Staphylococcus aureus</i>	TCC/24	12.7±0.3	14.3±0.6	12.3±0.3	18.7±0.3	14.0±0.6
<i>Escherichia coli</i>	Amx/21	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<i>Shigella flexneri</i>	Te/20	11.0±0.6	13.0±0.6	11.7±0.3	16.7±0.3	13.00±0.0
<i>Salmonella typhi</i>	Cip/26	9.3±0.3	10.0±0.0	8.3±0.3	12.3±0.3	9.0±0.6
S2005BAEEQ	Te/22	14.7.0±0.0	16.3±0.3	14.0±0.6	20.7±0.3	13.0±0.6
S 8250HI	Cip/27	11.7±0.3	13.3±0.0	11.3±0.3	15.7±0.3	11.0±0.57
SATCC29213	TCC/26	15.7±0.3	18±0.0	15.3±0.8	22.0±0.0	14.0±0.6
E2008IAEEQ	Amx/22	8.0.0±0.0	8.7±0.0	8.67±0.3	11.0±0.6	8.0±0.6

Ar: Reference antibiotic, Te: Tetracycline clavulanic acid, Cip: Ciprofloxacin, Amx: Amoxicillin, CR: Control, EAT: Aqueous terminalia extract; EMT: Methanolic extract of terminalia, EMTF2: Dichloromethane fraction, EMTF3: Acetate fraction and EMTF4: Methanolic residue

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration

Bacteria	Methanolic extract			Aqueous extract		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
<i>Shigella flexneri</i>	12.5	25	2	6.25	12.5	2
<i>Salmonella typhi</i>	25	50	2	6.25	12.5	2
<i>Staphylococcus aureus</i>	6.25	6.25	1	6.25	6.25	1
<i>Shigella flexneri</i> . 2005 BA EEQ	6.25	12.5	2	3.12	6.25	2
<i>Salmonella typhi</i> 8250H	12.5	25	2	3.12	6.25	2
<i>Staphylococcus aureus</i> ATCC 29213	6.25	6.25	1	3.12	6.25	1

MIC: Minimum inhibitor concentration (mg/mL) and MBC: Minimum bactericidal concentration (mg/mL)

(Table 3). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined (Table 4).

## DISCUSSION

Preliminary phytochemical screening shows the presence of phenolic compounds but no alkaloids. Musa *et al.*<sup>10</sup> noted the presence of alkaloids in the plant collected in Northern Nigeria. These authors found that the roots and bark of the trunk have the same chemical constituents as the leaves. Mann *et al.*<sup>8</sup> did not demonstrate the presence of alkaloids in the plant collected in Bida in Central Nigeria and that the leaves do not contain saponins, unlike the bark. This variation in the composition of secondary metabolites of *Terminalia avicennioides* may be linked to climate or soil. In all environments, the plant is very rich in phenolic compounds such as flavonoids and tannins. The chromatographic analysis of the methanolic extract of the leaves revealed that the phenolic compounds were derived from gallic acid.

Several studies have shown the antimicrobial activity of extracts from the bark of *Terminalia avicennioides*. The vibrocidal activity in cholera, antibacterial (on *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoea* and *Candida albicans*) were reported<sup>14</sup>.

The aqueous and methanolic extracts of the leaves of *T. avicennioides* have an inhibitory power on the growth of bacteria of the *Salmonella* and *Staphylococcus* genus but showed no effect on the clinical strain of *E. coli*. This activity may be linked to phenolic compounds. The gastro-protective action of phenols such as gallic acid came from their ability to reduce the surface area of gastric lesions; the tannins show excellent antiparasitic activities and can be used to limit excessive secretions such as diarrhea<sup>22</sup>.

Tannins are also capable of forming an insulating and protective coagulation layer for mucous membranes and tissues and would contribute to the treatment of gastric problems. Methylgallate plays an important role in the treatment of bacterial infections by disrupting the homeostasis of bacterial proteins, destroying the integrity of the bacterial membrane and enhancing the antibacterial effect of antibiotics<sup>23</sup>. The inactivity of the hexanic fraction on the bacteria studied is a consequence of the insolubility of the phenolic compounds in this solvent. This was confirmed by the more marked reactivity of the ethyl acetate fraction which is a more polar solvent. The difference in sensitivity between clinical strains and reference strains can be explained by the chemoresistance developed by wild germs in perpetual mutation.

## CONCLUSION

Gallic acid derivatives constitute the main phenolic group in *Terminalia avicennioides* leaves. These compounds have anti-inflammatory and healing properties which justify the use of the plant in traditional medicine in the treatment of wounds and gastric ulcers. The antioxidant power of these phenolic compounds favors the use of this plant in the preparations of improved traditional medicines. Although the extracts of this plant have no effect on *Escherichia coli*, they have an antibacterial effect on *Staphylococcus aureus*, *Salmonella typhi* and *Shigella flexneri* which are bacteria implicated in diarrhea in Niger. The presence of tannins in *Terminalia avicennioides* supports traditional medicinal use as an antidiarrheal and wound healer.

## SIGNIFICANCE STATEMENT

The phytochemical screening and the antimicrobial activity of the leaf extracts from *Terminalia avicennioides* were studied. Phytochemical screening of the extracts revealed the presence of phenols, tannins, flavonoids and steroids. The dosage of phenolic compounds showed an abundance of phenols, flavonoids and tannins. These phenols are derivatives of gallic acid: Methyl gallate, Galloylquinic, Galloyl glucose and Ellagic acid. The aqueous and methanol extracts showed moderate antibacterial activity against some clinical and reference bacteria strains such as *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus* but no effects on *Escherichia coli*. The ethyl acetate fraction showed better antibacterial activity. These results suggest that *Terminalia avicennioides* can be used in a therapeutic preparation as antibacterial, astringent and antioxidant.

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