

MACROSCOPICAL CHARACTERS AND PHYTOCHEMICAL ANALYSIS OF LEAVES OF *LYCOPERSICON ESCULENTUM* MILL. AND ITS ANTIMICROBIAL ACTIVITY.

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Abstract

Lycopersicon esculentum Mill., Myanmar name Khayan-gyin, belongs to the family Solanaceae. The specimens were collected from surrounding area of Nampha Inn, Banmaw Township in Kachin State during the flowering and fruiting period. The collected plants were identified by the literature references to confirm its identity. The present research was carried out to study the macroscopical characters, phytochemical analysis and antimicrobial activity of the leaves of *Lycopersicon esculentum* Mill. For the phytochemical analysis, the leaves powder was investigated to determine the presence or absence of active chemical constituents according to the methods of Marini Bettalo and Trease and Evans. For the antimicrobial experiment, the seven solvent extracts of powdered leaves were tested on six microorganisms by using agar well diffusion methods. In the result of macroscopical studies, inflorescences were extra-axillary cymes and flowers were hypogynous. In qualitative analysis, alkaloids, glycosides, flavonoids, α -amino acids, reducing sugar, carbohydrates, protein, steroids, terpenoids, starch and saponins were found to be present in the leaves of *L.esculentum* Mill. In the results of antimicrobial studies, aqueous extract of leaves showed the highest activity on all six microorganisms. Therefore, the leaves of *L. esculentum* Mill. may serve as a source of natural antimicrobial agent to be used in medicinal purposes.

Key words: *Lycopersicon esculentum*, phytochemicals, Antimicrobial activity

Introduction

Medicinal plants are important for pharmacology researches and drugs development. The phytochemical is not only used directly as therapeutic agents, but also employ as preexisting materials for the synthesis of drugs or the models of pharmacological active compounds (Rendle, 1952). A medicinal plant *Lycopersicon esculentum* Mill. belongs to the family solanaceae. The specimens were collected from surrounding area of Nampha Inn, Banmaw Township in Kachin State. Solanaceae is a family of about 94 genera and more than 2950 species. It is distributed in most part of the world, more especially in tropical America (Mabberley, 1987). *Lycopersicon esculentum* Mill is an important vegetable crop grown mainly for its edible fruits and sometimes leaves. As a popular vegetable crop, it ranks second in importance to the potatoes in many countries (Heywood, 1982). The leaves of *L.esculentum* are boiled and applied as poultices to relieve pain while the leaves are pulped for embrocation area of local plain. The infusion of the leaves is also used in the treatment of gonorrhoea. The leaves are considered antibiotic and fungicidal on sore (Debjit Bhowmik, 2012).

In the present study, macroscopical characters, preliminary phytochemical investigation and antimicrobial studies had been undertaken. As a result, the leaves of *L.esculentum* revealed the presence of important active constituents and antimicrobial properties. Thus the leaves of *L.esculentum* Mill. may serve as a source of natural antimicrobial agents to be used in food and medicinal systems.

Therefore, the aim of this study are to find the medicinal plant scientifically which has effective medicinal values , to investigate the active chemical constituents of the leaves of medicinal plant and to find out the highest activity of leaves extracts on six pathogenic microorganisms.

Materials and Methods

Macroscopical study

The specimens of *Lycopersicon esculentum* Mill. used in this study were collected from surrounding area of Nampha Inn, Banmaw Township in Kachin State. For the identification of their macroscopical characters, the vegetative and reproductive parts of the plant were collected at their flowering period to fruits and seeds.

Classification and identification were done with the help of available literature cited in Adelanwa et.al; (2011), Backer (1965), Dassanayake (1987), Hooker (1979), Hu Qi-ming (2009), Jagatheeswari (2014), Kress et.al., (2003), and Yadav et.al.,(2006).

After the collection, both the vegetative and reproductive parts of the fresh specimen were measured and recorded for taxonomic description. And then, photographs of the all parts of studied specimens were taken. The collected specimens were properly dried, pressed and mounted on the herbarium sheets. Moreover, these dried specimens were crushed and pounded into powdered form. This powder was stored in the airtight container for another study.

Phytochemical Study

The preliminary phytochemical investigation on the powdered leaves of *L.esculentum* Mill. were carried out to determine the presence or absence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, α -amino acids, starch, reducing sugar, saponins, tannins, carbonhydrates and protein. The methods of Marini Bettalo (1981) and Trease and Evans (2002) were applied for investigation of phytochemicals. These experiments were carried out at the Department of Botany, Banmaw University.

Antimicrobial Study

Extraction and examination of antimicrobial activity

The dried powder sample of leaves was extracted with pet-ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water. The various solvents extracts of leaves were tested on six pathogenic microorganisms such as *Bacillus pumalis*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These experiments were carried out at Central Research and Development Centre (CRDC).

The study of antimicrobial activities was performed by using agar-well diffusion method according to Cruickshank (1975). Nutrient agar was prepared and boiled, and then 20-25 ml of the medium was poured into a test-tube and plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the tubes were cooled down at (30-35°C) and the medium was poured into sterilized petridishes and 0.1 – 0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2-3 hours. After this, 10mm agar well was punched with the help of sterilized cork borer. After that, about 0.2ml of sample was introduced into the agar well and incubated at 37°C for 24 hours. The inhibitory zone appeared around the agar well, indicating possesses of antimicrobial activity. Then, the diameter of inhibitory zone including 10 mm agar well were measured with the help of a transparent ruler.

Similarly, the controlled experiments using solvent only were prepared for the comparison with plant extracts.

Results

Scientific Name	-	<i>Lycopersicon esculentum</i> Mill
Myanmar Name	-	Khayan-gyin
English Name	-	Tomato
Family	-	Solanaceae (Night-Shade Family, Potato Family)
Flowering and Fruiting Period	-	Summer to Autumn
Useful Parts	-	Fruits, leaves, roots

Macroscopical Characters of *Lycopersicon esculentum* Mill

Lycopersicon esculentum Mill. commonly known as the tomato plant is an edible, annual, fetid-herbs, attaining a height of 0.5 – 1.2m long; stems and branches terete, solid, coarsely hairy and glandular. Tap root is strong with a dense system of lateral and adventitious roots. Leaves alternate, imparipinnate compound; lamina 2.0-9.0 cm long and 2.1-6.0 cm wide; upper surface dark green and lower surface pale green; both surfaces sparsely pubescent, petiolate, 1.3-2.0 cm long and 0.1-0.3 cm wide; exstipulate; leaflets ovate or ovate-oblong, 1.7-2.5cm long and 1.2-1.8 cm wide, margin sinuately toothed; oblique at the base, acute at the apex, sparsely pubescent on both surfaces. Inflorescences extra-axillary cymes with 2-8 flowers, pedicel 0.4-0.7cm long and 0.1-0.2cm wide; Flowers yellow, about 2.8-3.3 cm long at anthesis, ebracteate, ebracteolate, complete, bisexual, regular, actinomorphic, 5-6 merous; pedicellate, hypogynous. Calyx 5-6 lobed, persistent; tube 0.2-0.3 cm long and 0.4-0.5 cm wide; lobes lanceolate, green and enlarging on fruit, lobes 1.1–1.2 cm long and 0.1-0.3 cm wide; stellately and glandular-hairy inferior. Corolla rotate, yellow, 5-6 lobed; 1.3-1.8cm long and 0.5-0.8cm wide; and tubes 0.3-0.5cm long and 0.6-0.8cm wide, both surfaces sparsely pubescent, lobes becoming reflexed, caducous, inferior. Stamens 5-6, epipetalous, adnate to the corolla tube. Filament short; 1-1.2cm long; anthers ditheous, coherent, oblong, bright yellow, introrse, basifixed, longitudinally dehiscence, inferior. Ovary globoid, oblique, glabrous, 0.3 cm long and 0.2cm wide, superior, monocarpellary, syncarpous, 2-3 locular with many ovules in each locule in T.S; axile placentation, style filiform; 1-1.3 cm long, stigma capitate, pale-yellow. Fruit a berry, globular to oblate, smooth or furrowed, green and hairy when young, glabrous and shiny, usually red, sometimes oranges or yellow when ripe, 3.6-5.5 cm long and 3.2-4.5 cm wide. Seeds numerous, 0.3-0.4 cm long and 0.2 cm wide, flattened rounded or ovoid, pale yellow and hairy, slightly pitted, smooth, endospermic.



Fig 1. Habit of *Lycopersicon esculentum* Mill.

- A. Habit
- B. Root
- C. Leaves (Upper surface)
- D. Leaves (Lower surface)
- E. Inflorescence
- F. Young floral buds

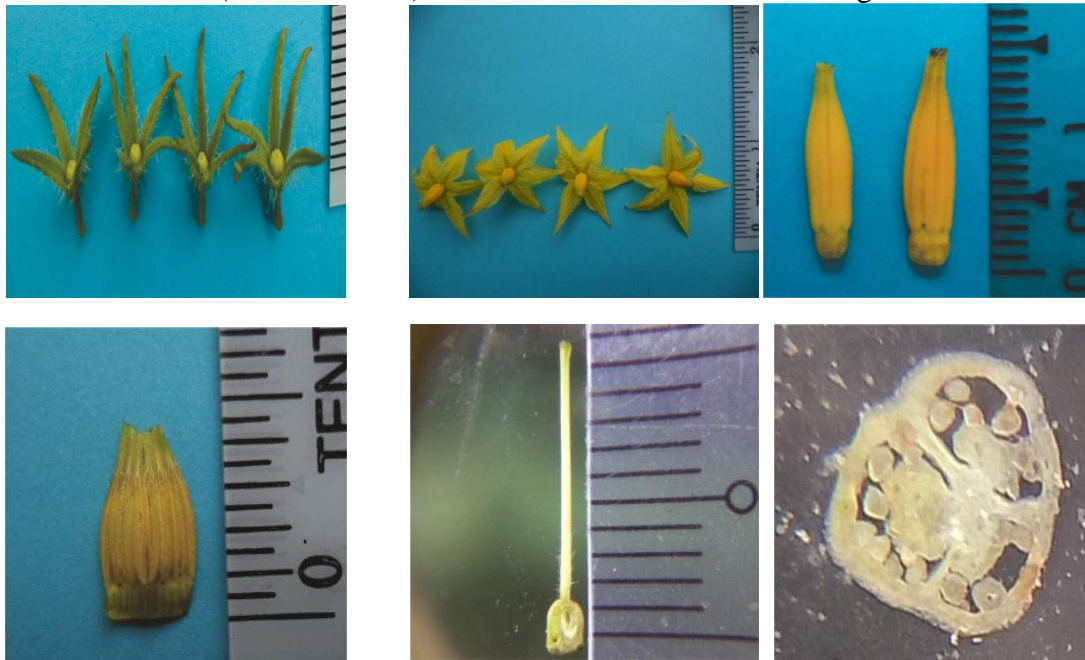


Figure 2. Floral Parts of *Lycopersicon esculentum* Mill.

- A. Calyx
- B. Corolla lobes
- C. Androecium
- D. Anther
- E. Gynoecium
- F. T.S of Ovary

Preliminary phytochemical investigation of leaves of *Lycopersicon esculentum* Mill.

The preliminary phytochemical investigation was carried out on the powdered leaves.

Table (2) Preliminary phytochemical test of leaves of *Lycopersicon esculentum* Mill

No	Constituents	Extract	Test Reagents	Observation	Remark
1.	Alkaloids	2% HCL acid + EtoH	<ul style="list-style-type: none"> • Mayer's reagent • Hager's reagent • Wagner's reagent 	White ppt Yellow ppt Reddish brown ppt	+
2.	Glycosides	Ethanol	1 ml of water and sodium hydroxide	Yellow Colour	+
3.	Phenolic compounds	H ₂ O	3% Ferric chloride solution	Green Colour	+
4.	Flavonoids	Ethanol	Small pieces of Mg, few drops of HCl	Pink Colour	+
5.	Steroids	Pet-ether	Acetic anhydride and Conc: H ₂ SO ₄	Green	+
6.	-amino acids	H ₂ O, spotted on filter paper	Dry and sprayed with Ninhydrin reagent and kept in over at 110C	Pink Spot	+
7.	Terpenoids	CHCl ₃	Acetic anhydride and Conc: H ₂ SO ₄	Pink	+
8.	Starch	H ₂ O	Iodine Solution	Bluish black ppt	+
9.	Reducing sugar	H ₂ O	Benedict Solution	Brick red ppt	+
10.	Saponins	H ₂ O	Distilled Water	Frothing	+
11.	Tannins	H ₂ O	5% Ferric Chloride slouton and sulphuric acid	No yellowish brown ppt	-
12.	Carbohydrates	H ₂ O	1 ml of a mixture of equal parts of felling's solution A and B	Brick red ppt	+
13.	Protein	H ₂ O	NaOH Sol: and 3% CuSO ₄ Sol:	Red or violet colour	+

(+) Present, (-) Absent

The tests indicated that, alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, -amino acids, starch, reducing sugar, saponins carbohydrates and protein were found to be present and tannin was absence in the leaves of *Lycopersicon esculentum* Mill.



Figure 3. Phytochemical investigation of leaves of *Lycopersicon esculentum* Mill.

Antimicrobial activity of various solvent extracts of leaves of *Lycopersicon esculentum* Mill.

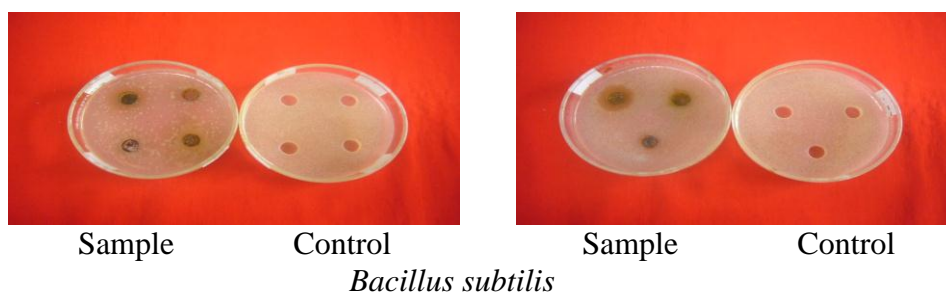
Antimicrobial activity of various solvent extracts such as pet-ether, chloroform, ethyl acetate, acetone, ethanol, methanol and aqueous extract were tested on six microorganisms. The results were shown in Table (2) and Figure (4).

Table (2) Antimicrobial activity of various solvent extracts of leaves of *Lycopersicon esculentum* Mill.

Smapple	Solvent	<i>B.Subtilis</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>B.pumalis</i>	<i>C.albicans</i>	<i>E.coli</i>
Leaves	Pet-ether	20mm	-	-	-	-	12mm
	CHCL ₃	-	-	-	-	13mm	12mm
	MeOH	-	13mm	-	13mm	-	13mm
	Acetone	-	-	-	14mm	-	13mm
	EtOAc	13mm	15mm	12mm	15mm	15mm	15mm
	EtOH	12mm	14mm	13mm	14mm	13mm	13mm
	Water	21mm	22mm	26mm	22mm	24mm	24mm
Control	Pet-ether	-	-	-	-	-	-
	CHCL ₃	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
	Water	-	-	-	-	-	-

Agar well (10)mm

In this experiment, aqueous extract of leaves showed the highest activity especially more sensitive against *Pseudomonas aeruginosa* (26mm); secondly sensitive against *Candida albicans* and *E.coli* (24mm); thirdly sensitive against *Staphylococcus aureus* (22 mm), *Bacillus pumalis* (22 mm) and *Bacillus subtilis* (21 mm). Pet-ether extract of leaves showed the highest activity especially more sensitive against *Bacillus subtilis* (20 mm).



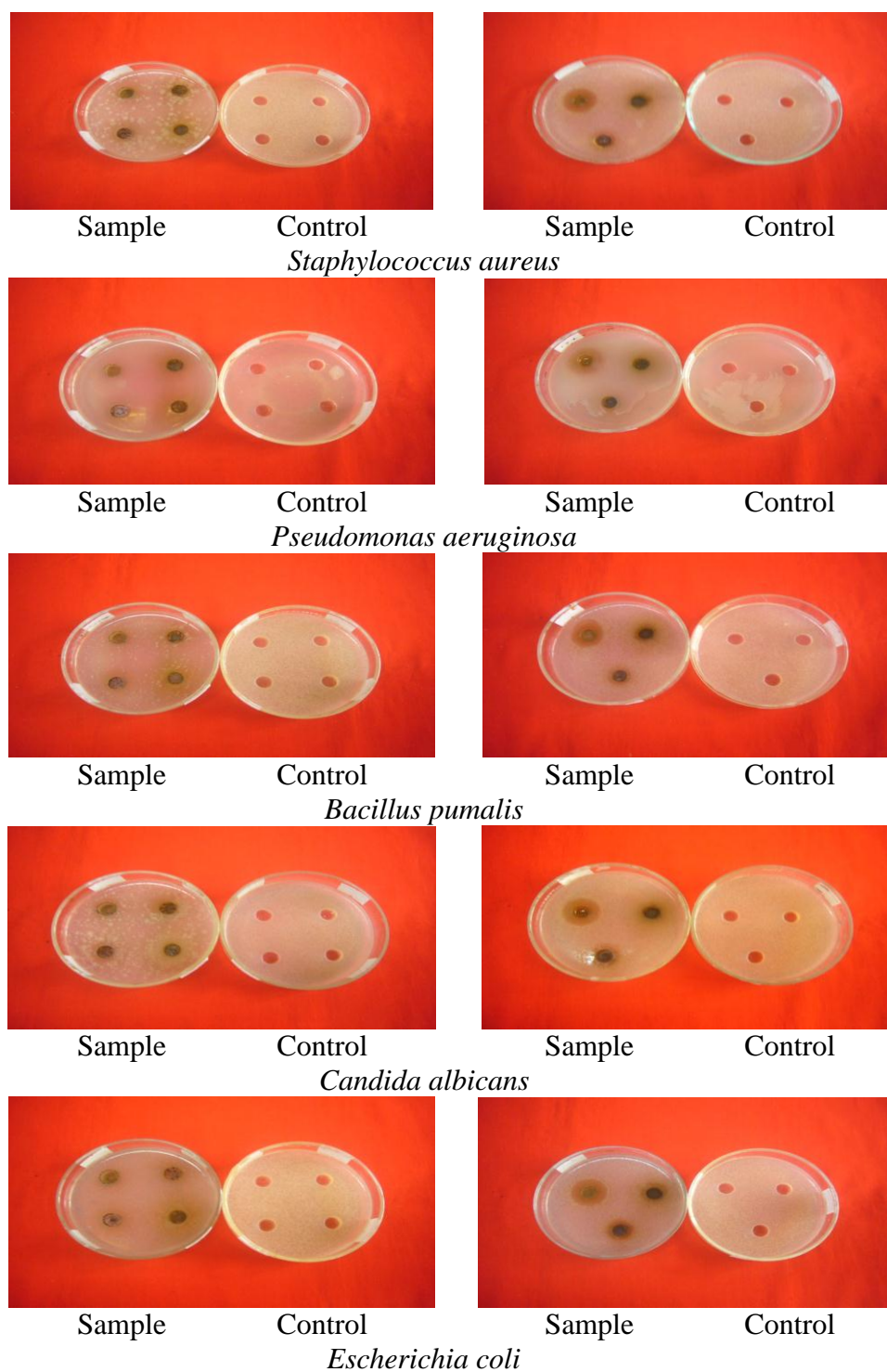


Figure 4. Treatment of various extracts from the leaves of *Lycopersicon esculentum* Mill.

Discussion and Conclusion

A medicinal plant *Lycopersicon esculentum* Mill. belongs to the family Solanaceae. The specimens were collected from surrounding area of Nampha Inn, Banmaw Township in Kachin State. In the present investigation, the macroscopical studies on both vegetative and reproductive parts of the plant, preliminary phytochemical analysis and antimicrobial activity of the leaves had been undertaken ..

As a result of macroscopical studies, the plant of *L. esculentum* Mill. was annual, fetid-herbs, stems and branches terete, solid, coarsely hairy and glandular. Leaves were alternate, imparipinnate compound; exstipulate, petiolate, both surfaces of lamina sparsely pubescent; leaflets were ovate or ovate-oblong, margin sinuately toothed; oblique at the base, acute at the apex. Inflorescences extra-axillary cymes with 2-8 flowers; pedicellate. Flowers were yellow, ebracteate, ebracteolate, bisexual, actinomorphic, 5-6 merous; hypogynous. Sepals 5-6 lobed, persistent, lobes lanceolate, stellately and glandular-hair. Petals 5-6 lobed, rotate, both surfaces sparsely pubescent, caducous. Stamens 5-6 lobed, epipetalous; anther ditheous, introse, basifixed. Ovary oblique, glabrous, monocarpellary, axile placentation. Fruit a berry, seeds numerous, flattened rounded or ovoid, endospermic. These characters are in agreement with those mentioned by Admin (2017), Backer (1965), Dassanayake (1987), Hooker (1879), HU Qiming (2009), Jagatheeswari (2014), Kress et al (2003), and Yadav (2016).

The preliminary phytochemical investigation was carried out on the powdered leaves. These tests indicated that alkaloids, glycosides phenolic compounds, flavonoids, steroids, terpenoids, α -amino acids, starch, reducing sugar, saponins, carbohydrates and protein were found to be present; tannin was absent in leaves of *L. esculentum* Mill. According to Etxeberria (2006), phytochemical analysis of leaves of this plant revealed presence of alkaloids, glycosides, flavonoids, steroids, terpenoids and carbohydrates. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer and help to stop carcinogens from attacking cells.

The antimicrobial activity of various solvent extracts such as pet-ether, chlorform, ethyl acetate, acetone, ethanol, methanol and aqueous extract were tested on six microorganisms. In this experiment, aqueous extract of leaves showed the highest activity especially more sensitive against *Pseudomonas aeruginosa* (26mm), secondly sensitive against *Candida albicans* and *Escherichia coli* (24mm) and then thirdly sensitive against *Staphylococcus aureus* (22 mm), *Bacillus pumalis* (22 mm) and *Bacillus subtilis* (21 mm). Pet-ether extract of leaves showed the highest activity especially more sensitive against *Bacillus subtilis* (20mm). Ethyl acetate extracts of leaves showed the highest activity on *Staphylococcus aureus*, *Bacillus pumilus*, *Candida albicans* and *E.coli* (15 mm).

From this finding, it can be inferred that leaves of *L. esculentum* Mill. can be effective in the formulation of medicine for the treatment of disease caused by *B. subtilis*, *S. aureus*, *P. aureginosa*, *B. Pumalis*, *C. albicans* and *E. coli* such as wound infection, pneumonia, urinary tract infection, respiratory system infection, soft tissue infections, ear infection, eye infection, skin infection, vaginal candidiasis, diarrhea and dysentery.

Therefore, the results of this present study on macroscopical characters can give a few information on the systematics study on a member of the family solanaceae. Moreover, the leaves of *L. esculentum* contained many chemical constituents. They are employed for medicinal purposes. Finally, the leaves of *L. esculentum* Mill. may serve as a source of natural antimicrobial agents to be used in food and medicinal

system. The main objective of the present research work was that *L.esculentum* had medicinal values not only in fruit but also in leaves.

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