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Study on the Pharmacognosy of *S. Spatuliforius*

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Abstract: *Sauropus spatulifolius* Beille (*S. spatulifolius*) is the dried plant of the genus *Sauropus,* and belongs to the family Euphorbiaceae. To study the pharmacognosy of *Sauropus spatulifolius* Beille (*S. spatulifolius*). A preliminary analysis in terms of primal identification, macroscopic identification and chemistry was carried out on *S. spatulifolius*. The transverse section of *S. spatulifolius* roots and stem showed that the cambium was obvious and the xylem was well developed; the transverse section of roots shows that the phloem appeared narrow and the vascular bundle was collateral. The transverse section of the stem showed occasional prickling of the periderm. The transverse section of the leaves showed that the lower epidermal cells are covered by non-glandular hairs; the palisade cells and spongy tissues were clearly distinguished; the stomatic type of the lower epidermis was paracytic, with stomatal index of 31.76%. Fiber, scalariform vessels and spiral vessels, pollen grains and so on were commonly seen in the powder. Through chemical identification, polysaccharides, glycosides, alkaloids and other components were identified in the plant. TLC result shows that T-test result was 0.018, indicating significant difference. The pharmacognostic features of *S. spatulifolius* are obvious, providing reference for further utilization and development of this plant.

Keywords: *Sauropus spatuliforius* Beille, pharmacognosy, microscopic identification, physical, chemical identification.

INTRODUCTION

Sauropus spatulifolius Beille (*S. spatulifolius*) is the dried plant of the genus *Sauropus,* and belongs to the family Euphorbiaceae. It is also known as Dragon's Tongue Leaf, Long Li Ye, Long Wei Ye or Long Feng Ye in Chinese, and is a perennial evergreen undershrub. *S. spatulifolius* is the native of Sumatra, Indonesia and is now widely distributed in southern China (Ma and Liu, 2016). It is usually cultivated in Guangdong and Guangxi provinces and also grown in Fujian, Yunnan, Jiangxi, Hainan and Hong Kong. Due to its small plant body, it is often planted in the medicinal garden, park or near the village and the house.

S. spatulifolius is the only medicinal plant in the genus *Sauropus*, but is not been widely studied and mostly used by folk people. *S. spatulifolius* is often used as a soup material in daily life, which helps to resolve phlegm and relieve cough for treating asthma (Ding and He, 2015). The leaves of *S. spatulifolius* can be used as a medicine. It is a natural, sweet and bland that clears the heat and moistens the lung by resolving the phlegm and relieving the cough, and also moisturizes the intestines to relax the bowel (Commission, 2015). It is mainly used to treat symptoms like lung heat and phlegm or cough with abundance of phlegm, bronchial asthma, acute bronchitis, upper respiratory tract inflammation, aphonia, tuberculosis, sore throat, dry mouth and constipation. Modern studies have shown that the extracts from *S. spatulifolius* also have antimalarial, antioxidant and anti-inflammatory effects (Mo, 2016). But there are few systematic studies on the pharmacognosy of S. spatulifolius. Hence, our study investigated the pharmacognostic features of *S. spatulifolius* to provide the basis for further development and utilization.

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MATERIALS AND INSTRUMENTS

Materials and reagents

Fresh plants of *S. spatulifolius* were collected from Guangzhou Higher Education Mega Center and identified it as the whole plant of *S. spatulifolius* of the genus Sauropus, and family Euphorbiaceae. Dried plants of *S. spatulifolius* were purchased from the farmer's market in Zhaoqing, Guangdong and identified it as a whole plant of the genus Sauropus and family Euphorbiaceae by Professor Ji Shengguo.

FAA stationary test solution (formaldehyde-acetic acid-ethanol mixture), diluted glycerol test solution, chloral hydrate test solution, 10% α-naphthol ethanol reagent, concentrated sulfuric acid solution, ninhydrin reagent, 1% $FeCl_3$ reagent, 10% NaOH solution, acetic anhydride, bismuth potassium iodide, I-KI, silicotungstic acid and phosphomolybdic acid were prepared according to the Appendix XVB of Volume I in the *Pharmacopoeia of the People's Republic of China 2015*.

2g of *S. spatulifolius* powder was taken, added 50mL water, and applied ultrasound for 30min. Then the solution was filtered, the filtrate was evaporated and 1mL of methanol was added to the residue for dissolving it and this was considered as the sample solution. The experiment was carried out by thin-layer chromatography (Appendix VI B). The sample solution of 10μL was taken

as test and poured on the same silica gel G plate. Chloroform-acetone-formate (6:6:1) was used as the developing solvent. The solvent was then developed (temperature 21°C, relative humidity 31%), dried and placed it under the ultraviolet lamp (302nm) for inspection.

Instruments

E100 Biomicroscope (Nikon);
DS-8510DT Ultrasonic Cleaner (Shanghai Sonxi Ultrasonic Instrument Co., Ltd.);
ZF-8 Camera Obscura Four-purpose UV Analyzer (Shanghai Jiapeng Technology Co., Ltd.);
DE-100g Universal High Speed Grinder (Shanghai Jiangxin Technology Co., Ltd.);
H1650-W table model high speed centrifuge (Hunan Xiangyi Laboratory Instrument Development Co. Ltd.);
Electrophoretic apparatus trophoresis (Beijing Baijing Biotechnology Co. Ltd.);
TR-402 Electronic balance (Beijing sartorius Instrument System Co. Ltd.).

RESULTS

Identification of origin

The plant is a perennial evergreen undershrub, 10-40cm tall, with rough stem, cylindrical branches of 2-5 mm in diameter, meandering bends, rugose and glandular-pubescent when young, glabrous when growing older, short internodes of 2-20mm long. Leaves are usually crowded on the upper part of the branchlets, often bent downward, alternate simple leaves, fresh leaves that are nearly succulent, and dried leaves of nearly leathery or thick paper texture, spatulate, obovate-oblong or ovate, sometimes oblong, 4.5-16.5cm long, 2.5-6.3cm wide, apex rounded or obtuse, mucronate, rarely concave, base cuneate or obtuse, rarely orbicular, dark green on the upper surface of fresh leaves and grayish white at the vein and the grayish white at the vein gradually becomes grayish green and glabrous when dried; sometimes glandular-pubescent at the base of the lower surface, and glabrous when becomes mature; midrib and lateral vein appeared flat in the fresh leaves, both sides of the midrib were raised when dried, 6-9 lateral veins on each side, slightly raised below; the petiole is 2-5 mm long, initially glandular-pubescent and later glabrous; stipules were triangular-auriculate, inserted on both sides of the petiole base, 4-8 mm long, base of 3-4 mm wide and persistent.

The blooming period is from February to October each year. The plant has red or purplish red flowers, monoecious, 2-5 flowers clustered in the middle or lower part of the deciduous branches, sometimes cauliflory, forming short cyme of up to 15 mm. The peduncle is short and robust, bearing many lanceolate bracts of ca. 2 mm long. Male flowers: These include filamentous pedicel, with 3-5mm long; 6 sepals, 2-whorled, nearly isometric, obovate, 2-3mm long, ca. 1.5mm wide; 6 flower disc glands, antesepalous; 3 stamens, filaments united in short columnar. Female flowers: These include pedicel ca. of 2-3 mm long; sepals identical to those of male flowers; have no flower disc; ovary subglobose, ca. of 1mm in diameter, 3 chambers, apex 2-whorled (fig. 1A & 1B).

Fig. 1: A Original plant and flower of *Sauropus spatuliforius* Beille B Flower and fruit of *Sauropus spatuliforius* Beille

Fig. 2: Dried sauropus *Sauropus spatuliforius* Beille

Macroscopic identification

The leaves of *S. spatulifolius* were used as medicine. It is crisp, and nearly has paper texture, agglomerated or elongated shrinkage, long-oval, ovate-lanceolate or obovate-lanceolate after flattened, yellowish brown,

yellowish green or greenish brown surface, 5-9cm long and 2.5-3.5cm wide. The main and primary veins were greyish green and the secondary vein is unobvious. The leaf apex is obtuse and slightly concave with small spines, base cuneate or slightly rounded and the plant completely or slightly crumpled into wavy shape. The midvein on the lower surface protruded front and back, the base is occasionally pubescent, lateral vein pinnate with 5 to 6 pairs, synthesizing the marginal vein near the outer margin. The petiole is short(fig. 2).

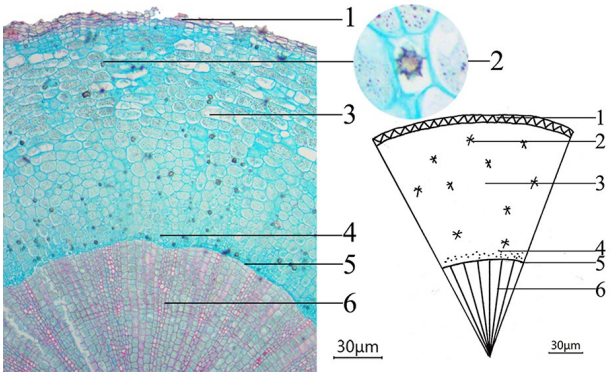

Fig. 3: Transverse section of root. 1PL.phellem layer; 2Cc. calcium oxalate cluster crystals; 3Co. cortex; 4Ph. phloem; 5Ca. cambium; 6Xy. xylem.

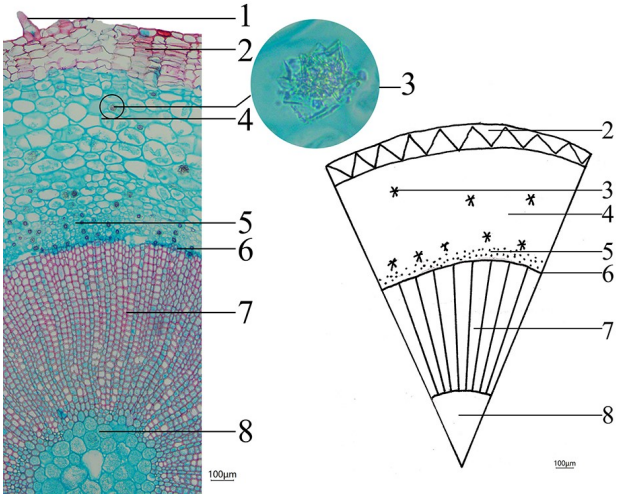

Fig. 4: Transverse section of stem. 1 Pr. Prickle; 2 PL. phellem layer; 3Cc. calcium oxalate cluster crystals; 4Co.cortex; 5Ph.phloem; 6Ca. cambium; 7Xy. xylem; 8Me. medulla.

Microscopic characteristics

Characteristics of transverse section of root

The transverse section of the roots of *S. spatulifolius* was nearly round, and the phellem layer composed of 5-8 layers of parenchymal cells and is closely arranged. The cortex is wide about 18~24 layers, the parenchymal cells are large and nearly oval in shape. Most of the parenchymal cells contain calcium oxalate clusters and starch grains. Complex granules are usually filled in single parenchymal cells. The cell volume was gradually decreased from outside to inside, with small intercellular space. The phloem was narrow, and the cells are polygonal and closely arranged. The cambium was obvious. The vascular bundle was collateral. The xylem was well developed and closely arranged in the form of rays, without intercellular space and strongly lignified. (fig. 3).

Characteristics of transverse section of stem

The transverse section of the stem appeared nearly round, and the periderm was occasionally shown with prickle. The phellem layer consisted of 5-6 layers of parenchymal cells. The cells are flat and arranged tightly. The volume of parenchymal cells in the cortex was gradually decreased from the epidermis to the cambium, and was nearly ellipsoid. There are about 13~15 columns, loosely arranged and contained calcium oxalate clusters and starch granules. The phloem was narrow, the cell was polygonal in shape, and there was no cell gap observed. The formation layer was obvious. The xylem was developed, and arranged radially. There was no space between the vessels and the vascular bundle appeared collateral. There was medulla in the middle, and consisted of parenchymal cells with a diameter of ca. 100μm and arranged loosely and the starch grains could be seen in the cells (fig. 4).

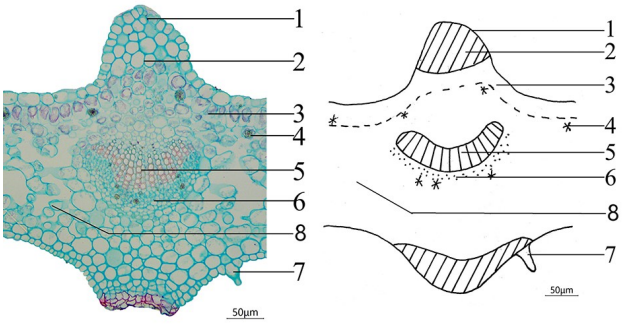

Fig. 5: Transverse section of leaves. 1Cu. cuticle; 2Co. collenchyma; 3PC. palisade cell; 4Cc. calcium oxalate cluster crystals 5Xy. xylem; 6Ph. phloem; 7NGH. non-glandular hair; 8St. spongy tissue.

Characteristics of transverse section of leaves

The upper and lower epidermal cells on the transverse section of leaves of *S. spatulifolius* had one layer of parenchymal cells. The lower epidermal cells are covered by non-glandular hairs. The parenchymal cells appeared after the main vein was slightly woody. The collenchymatous cells inside the upper and lower epidermal layers was about 2-4 layers. The palisade tissue cells appeared in one column, over the main vein and contained the calcium oxalate crystals. The palisade cells and the spongy tissues were distinguished clearly. The intercellular space of the spongy tissue remained large. The xylem in the vascular bundle was shallow groove type and arranged radially. The xylem was surrounded by

phloem cells, and closely arranged, containing a small amount of calcium oxalate crystals (fig. 5).

Leaf epidermal characteristics

The cells of both upper and lower epidermal layers are irregularly shaped and the anticlinal wall showed wavy bending. There were no stomata in the upper epidermis (fig. 6-A), and the stomata of lower epidermal layer were mostly paracytic (fig. 6-B). The two subsidiary cells were in different sizes, with a stomatal index of 31.76%.

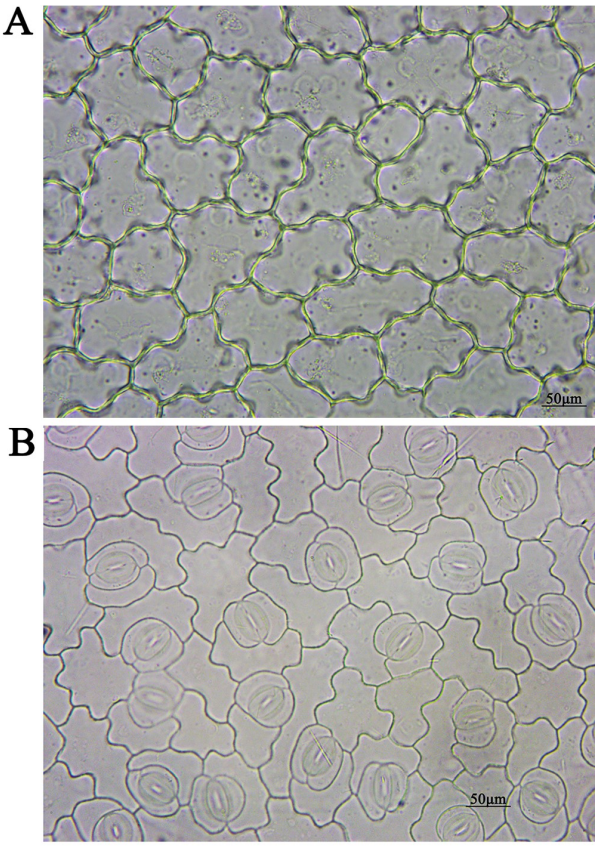

Fig. 6: Leaf epidermis of *Sauropus spatuliforius* Beille

Powder characteristics

Powder is grey green in color. 1. The fiber was found, the cell wall was thickened, the cell appeared tall, slender and straight, and the diameter was about 20-28μm; 2. annular vessels, scalariform vessels and spiral vessels of different sizes were seen everywhere and the diameter was about 15-30μm; 3. a large number of calcium oxalate cluster crystals were scattered, the angle was multi-end and blunt, with a diameter of about 30-40μm; 4. Pollen grains were sub-triangular, with verrucous protuberances on the surface, ca. 70μm in diameter (fig. 7).

Physical and chemical identification

Phytochemical screening

Preliminary qualitative phytochemical screening of water and petroleum ether soluble extract of *S. spatulifolius* showed the presence of sugar, polysaccharides, glycosides, amino acids, peptides, proteins, volatile oils and aliphatic acid. Ethanol extract showed the presence of flavones, while acid-water soluble extract showed alkaloids (table 1).

Physio-chemical analysis

Physio-chemical analysis revealed that the moisture content was 11.19%, total ash content was 3.99% and acid insoluble content was 0.86%, respectively (table 2).

Fluorescence analysis

Fluorescence analysis of powder and different extracts of *S. spatulifolius* with different reagents were carried out to observe the color reactions (table 3 & table 4).

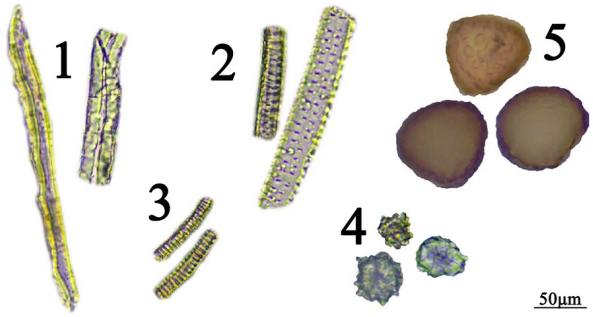

Fig. 7: Powder micrograph of *Sauropus spatuliforius* Beille. 1 Fi. Fiber; 2,3 Ca. catheter; 4 Cc. calcium oxalate cluster crystals; 5 Pg. Pollen grain.

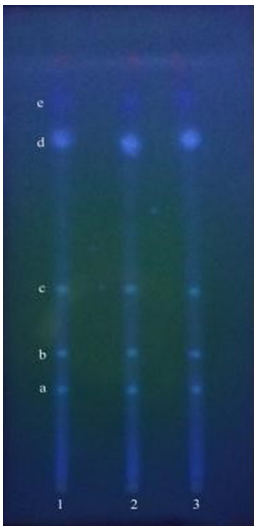

Fig. 8: Thin-layer analysis chromatogram of *Sauropus spatuliforius* Beille. 1, 2, 3 Sample solution for test.

Thin-layer chromatography

The results obtained were as follows: the samples tested showed blue spots at different positions of the silica gel plates and the Rf values of the five points, a, b, c, d and e were 0.20, 0.23, 0.35, 0.91 and 0.94, respectively (fig. 8). T-test showed that the result was 0.018, indicating significant difference.

Table 1: Preliminary phytochemical screening of *Sauropus spatuliforius* Beille

Phytochemicals	Test	Observations	Presence(+)/ absence(-)
Sugar, polysaccharide, glycosides	Molish test	The interface was purplish red	+ve
Amino acids, peptides, proteins	Ninhydrin test	Blue color observed	+ve
Saponins	Foam test	No foam generation	-ve
Tannin	Ferric chloride test	No obvious phenomenon	-ve
Volatile oils and fats	Filter paper test	There was oil stain on the filter paper, which disappeared after heating	+ve
Flavonoids	Hydrochloric acid-magnesium powder reaction	No obvious phenomenon	-ve
	Aluminum trichloride reaction	With yellow green color fluorescence	+ve
	Ammonia fumigation reaction	With yellow fluorescence	+ve
Anthraquinones	Alkali liquor test	No obvious phenomenon	-ve
Phytosterol, triterpenoids	Acetic anhydride-concentrated sulfuric acid reaction	No obvious change in color	-ve
Cardiac glycoside	Basic picric acid test	No obvious phenomenon	-ve
Alkaloids	Potassium bismuth iodide reaction	No obvious phenomenon	-ve
	Iodine-potassium iodide reaction	Reddish brown sediment	+ve
	Phosphomolybdic acid reaction	No obvious phenomenon	-ve
	Silicotungstic acid reaction	No obvious phenomenon	-ve

Note: "+ve" means that there is such a reaction, "-ve" means that there is no such reaction.

Table 2: Determination results of moisture and ash in *Sauropus spatuliforius* Beille

Item	Moisture	Ash	Acid-insoluble ash
Result (%)	11.19	3.99	0.86

Table 3: Powder phenomenon of *Sauropus Spatuliforius* Beille

Reagent	Glacial acetic acid	Sulphuric acid	Caustic potash	Caustic soda	Iron trichloride	Hydrogen nitrate	Hydrochloric acid
Solution color	Yellow	Brown	Yellowish green	Yellowish green	Green	Orange	Green
Powder phenomenon	Sediment	Carbonization	Sediment	Sediment	Floating	Floating	Floating

Table 4: Fluorescence phenomenon of *Sauropus spatuliforius* Beille

Reagent	In the sunlight	254nm	365nm
75% ethanol	Light green	Light pink	Light pink
Acetic ether	Green	Pink	Pink
Acetone	Dark green	Bright orange	Bright orange
Carbinol	Green	Light pink	Pink
Chloroform	Dark green	Light pink	Light pink
Carbon tetrachloride	Green	Light orange	Light pink
Distilled water	Orange	Light orange	No fluorescence
Petroleum ether	Light green	Light orange	Light pink

DISCUSSION

According to the report on microscopic identification of the stem of *S. spatulifolius* by Qiu Qin *et al.* (2016), the cortex contained more starch grains and fiber bundles, with narrow phloem, wide xylem, obvious ray and big medulla. Through microscopic identification of S. spatulifolius, Liu Rong (2009) found that the stomata on the leaf surface was shown only in the lower epidermis and was paracytic. The powder features mainly include spiral vessels, with non-glandular hairs. The conclusion of the above two reports are roughly consistent with that of our study. However, in this paper, there was no fibrous bundle seen in the cortex and no glandular hairs found in the powder. Non-glandular hairs were visible only in the transverse section of the leaf. A qualitative identification

of saponins and amino acids in the aqueous extracts from *S. spatulifolius* conducted by Chen Zhen (2014) showed that both ethyl acetate-formaldehyde-water (8:1.2:0.5) was used as the developing solvent and saponins adopted 10% sulfuric acid ethanol solution for color reaction, while amino acids adopted 0.2% ninhydrin ethanol solution for color reaction, with better effects. In this paper, chloroform-acetone-formate (6:6:1) was used as the developing solvent, and the degree of separation obtained was relatively good according to the standard thin layer chromatography (Appendix VI B). The previous studies of Shen Xiaojing (2013) on plants of genus *Sauropus*, the main compounds isolated from this genus included mainly organic acids, volatile oils, nucleosides and flavonoids. Jiang Jianguo *et al.* (2008) identified water extract, ethanol extract and petroleum ether extract of *S. spatulifolius* by chemical reaction method. It showed that the amino acids, polypeptides and proteins, sugars, polysaccharides and glycosides, saponins, tannins, organic acids, alkaloids, coumarins and lactones, volatile oil and grease are positive in the reaction phenomenon, while the others were negative in the reaction phenomenon. But in this paper, there was no positive reaction between saponins and tannins.

In conclusion, the transverse section of the root of *S. spatulifolius* showed that the parenchymal cells of the cortex mostly contained calcium oxalate clusters and starch grains; the phloem was narrow, and the cambium was seen obviously; the xylem was well developed and strongly lignified; and the vascular bundle was collateral. The transverse section of the stem showed that the periderm was occasionally prickled; the parenchymal cells in the cortex contained calcium oxalate clusters and starch granules; the cambium was obvious; the xylem was well developed; there was no space between the vessels and the vascular bundle was collateral; there was medulla in the middle, and contained starch grains in the cells. The transverse section of the leaves showed that the lower epidermal cells are covered by non-glandular hairs and the parenchymal cells after the main vein was slightly woody; the palisade tissue cells appeared in one column, i.e., over the main vein and contained calcium oxalate crystals; the palisade cells and spongy tissues were clearly distinguished; the intercellular space of spongy tissue was large; the xylem in vascular bundle was shallow groove type, and arranged radially; the xylem was surrounded by phloem cells, closely arranged and contained a small amount of calcium oxalate crystals; there were no stomata in the upper epidermal layer; the stomatic type of the lower epidermis was paracytic, with a stomatal index of 31.76%. Fiber, scalariform vessels and spiral vessels, pollen grains, calcium oxalate cluster crystals and columnar crystals were commonly seen in the powder. Through physical and chemical identification, polysaccharides, glycosides, amino acids, volatile oils, flavonoids, alkaloids and other components were found in *S. spatulifolius*.

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