Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(3): 456-461
Received: 05-04-2024
Accepted: 09-05-2024

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Sesquiterpene lactone in *Tapinanthus dodoneifolius* (Dancer) DC: A phytochemistry according parasite's host

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DOI: https://doi.org/10.22271/phyto.2024.v13.i3f.14987

Abstract
Loranthaceae are parasitic plants used in traditional medicine. Their use generally depends on the host plant. This shows that the biological properties of these species depend on their host. As phytochemistry is responsible for biological properties, it depends on the parasite's host. The aim of this study was to evaluate the variation in phytochemistry and antioxidant activity of *Tapinanthus dodoneifolius* leaves and bark harvested from different hosts using IR, UV and TLC methods. The results showed that the total polyphenol content of *T. dodoneifolius* twig bark harvested from *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum* was identical. The same observation is made with the leaves, the content of which remains lower than that twigs barks. Leaves contain more total flavonoids than twig bark. Infrared, UV and TLC analyzes showed the presence of sesquiterpene lactones in leaves extracts of *T. dodoneifolius* harvested from *A. lebbeck* and *P. reticulatum*. The best antioxidant activity was obtained with the bark of *T. dodoneifolius* harvested from *A. lebbeck* and *P. reticulatum*.

Keywords: *Tapinanthus dodoneifolius*, sesquiterpene lactones, host plant

Introduction
Loranthaceae are hemiparasitic phanerogams that attach themselves to the stems of woody plants and are responsible for much damage to their hosts [1]. Species belonging to the Loranthaceae family have shown metabolic fortifying activities [2].
Tapinanthus dodoneifolius (Danser) DC is a species of the Loranthaceae family that is used in traditional medicine for the treatment of various diseases. The biological activities of *T. dodoneifolius* depend on the host plant [1]. *T. dodoneifolius* is used for the treatment of hypertension [3] and to treat cancer and diarrhoea [4]. *T. dodoneifolius* has shown antidepressant properties [5]. *T. dodoneifolius* is used for its anticonvulsant properties [5]. The extract of this plant inhibits the growth of *Agrobacterium tumefaciens* [4]. According to Deeni and Sadiq [4], extracts of *T. dodoneifolius* have shown antimicrobial activity on some resistant bacteria and fungi isolated from animal husbandry. In veterinary medicine, this species is used to treat asthma, alergia, infertility and skin diseases [3]. *T. dodoneifolius* ethyl acetate and diethyl ether fractions of showed antiproliferative activity on cancer cells. Quercetin isolated from this species exhibited growth inhibitory, cytostatic effects and showed a broad spectrum of activities on several kinases overexpressed in the heart of certain cancers [6]. *T. dodoneifolius* showed a capacity to modulate the biological activity of neutrophils [6]. Studies have reported no toxicity effects in rats at 5000 mg/Kg [7]. *T. dodoneifolius* harvested from *Vittelaria paradoxa* CF Gaertn (Sapotaceae) showed numerous biological activities such as bronchorelaxant, hypotensive and vasodilator [8, 9]. These activities are due to the presence of a sesquiterpene lactone called dodoneine [10]. Sesquiterpene lactones are secondary metabolites that possess several pharmacological properties. According Ivanescu *et al.*, [11], sesquiterpene lactones have antibacterial, antifungal, antiparasitic and antihelminthic activities. Previous work has shown that sesquiterpene lactones have cytotoxic activities on cancer cell cultures [12, 13]. It is reported that sesquiterpene lactones (alantolactone, dehydrocostus lactone and costunolide) can be used in the treatment of inflammatory skin disorders [14]. The anticancer properties (pancreas, liver, breast, ovary, colorectal, etc.) have been widely reported [15]. isodeoxyelephantopin is a sesquiterpene lactone that has shown activity against breast cancer [16].
Sesquiterpene lactones have shown inhibitory activities on the production nitric oxide induced by lipopolysaccharide (LPS) [17, 18]. The aim of this work is to assess the variation in phytochemistry and antioxidant activity of *T. dodoneifolius* depending on its host.

Methods

The plant material used was *T. dodoneifolius* leaves and twig bark. *T. dodoneifolius* was harvested from three hosts, including *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum*.

Assessment of total polyphenols

The evaluation of total phenolics was performed according to the procedure described by Meda *et al*., [19]. To 25µL of extract (in water) is added 125 µL of FCR (0.2 N). 100 µL of a sodium carbonate solution (75g/L) was added 5 min after. The absorbance was read at 760 nm after one hour in the dark. The blank consisted of a mixture of 125 µL of FCR and 100 µL of sodium carbonate. The results are expressed as mg Gallic Acid Equivalent per 100mg dry extract (mgEAG/100mg).

Assessment of total flavonoids

Total flavonoids were assessed by Meda *et al*., [19]. A volume of 100 µL of 2% $AlCl_3$ (in methanol) is mixed with 100 µL of extract (0.1mg / mL, in methanol). For each sample, a blank was made by mixing 100 µL of extract and 100 µL of methanol. The absorbance was read at 415 nm after 15mn against a quercetin standard curve. The results are expressed as mg Quercetin Equivalent (QE) per 100 mg dry extract (mg QE/100 mg).

Antioxidant activity assessment

Antioxidant activity was assessed by scavenging the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical according to Velázquez *et al*., method [20]. To 0.1 mL of extract was added 0.2 mL of DPPH (20mg/L, in methanol). After incubation (15 minutes), the absorbance was read at 517 nm against a blank using a spectrophotometer (EPOCH BIOTEK USA). The control is composed of 0.1 mL of methanol and 0.2 mL of DPPH. A series of successive dilutions were made from 0.1mg /mL of extract (in methanol).

Identification of sesquiterpenic lactones.

The identification of sesquiterpenic lactones was done through several analysis methods including IR, UV and TLC.

IR analysis

Pellets made with 2% extracts (in KBr) were used. The IR spectra of the different extracts were recorded, using pellets with a Perkin Elmer Spectrum Bx Fourier Transform (FTIR) apparatus (application software: Spectra Manager). Scan are range from 400 to 4000 cm^{-1}.

Detection of sesquiterpene lactones by TLC

The detection was done according to Kringstad method [21]. The sesquiterpene lactones were revealed by two spraying reagents. Reagent 1 is prepared by mixing hydroxylamine hydrochloride solution (1.5M) and KOH solution (1.5M, in methanol) so as to obtain pH values ranging from 6 to 13. Reagent 2 was a solution of ferric chloride (0.037 M) in hydrochloric acid (2.7M). After development, the plate is heated to 105°C for one hour and then sprayed with reagent 1 (10 mL). After drying for 10 minutes at room temperature, the plate is sprayed with reagent 2 (3.5 mL). The appearance of the brown-purple or brown-violet colour immediately after spraying indicates the presence of sesquiterpenic lactone. The system constituted by chloroform-propan-2-ol-formic acid-water (2:8:2:1 v/v) was used as mobile phase.

UV analysis

Bark and leaf extracts were used at a concentration of 100 µg/mL. A volume of 200 µL was used for absorbance reading (200 to 800 nm). Methanol was used as the blank.

Statistical analysis: One way analysis of variance (ANOVA) followed by Tukey test of Graph Pad Prism software was used to determined statistical significance; p value ≤ 0.05 was considered significant (n=3).

Results

Total phenolic content

The total phenolic contents (figure 1) of *Tapinanthus dodoneifolius* bark extracts harvested on *Piliostigma reticulatum*, *Albizia lebbeck* and *Acacia seyal* respectively are 48.15±0.94 mgEAG /100mg, 44.72±0.91 mgEAG /100mg and 43.57±2.52 mgEAG /100mg. Regarding the leaves extracts, the total phenolic contents are 35.33±1.08 mgEAG /100mg, 30.34±0.33 mgEAG /100mg and 35.71±0.75 mgEAG /100mg for *T. dodoneifolius* respectively from *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum*. The results obtained show that the twig barks are richer in total phenolics than the leaves. The barks of the samples from the three hosts show statistically identical contents. The leaves of *T. dodoneifolius* from *Acacia seyal* and *Piliostigma reticulatum* show same content but that are higher than of leaves of *T. dodoneifolius* from *Albizia lebbeck.*

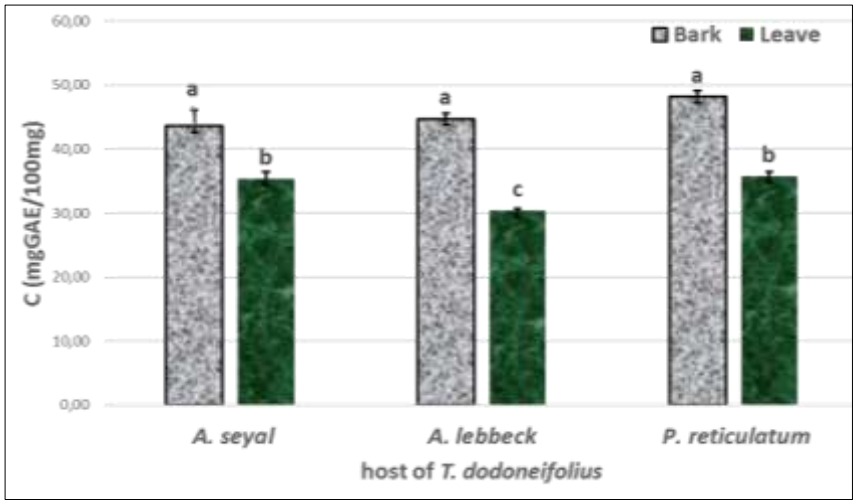

Fig 1: Total polyphenol content of methanolic extracts of leaves and bark of *T. dodoneifolius*. The results indicated by the distinct letters are statistically different ($p< 0.05$)

Total flavonoid content

Total flavonoids quantification results was presented in figure 2.

Bark total flavonoids content are 0.26±0.01 mgEQ /100mg, 0.15±0.06 mgEQ /100mg and 0.34±0.01 mgEQ /100mg respectively for *T. dodoneifolius* from *A. seyal*, *A. lebbeck* and *P. reticulatum*.

Leave total flavonoids contents are 1.14±0.02 mgEQ /100mg, 1.22±0.17 mgEQ /100mg and 1.27±0.29 mgEQ /100mg respectively for *T. dodoneifolius* from *A. seyal*, *A. lebbeck* and *P. reticulatum*.

Flavonoid contents of leaves are at least three times higher than those of twig barks. The contents of the leaves are statistically identical (p> 0.05).

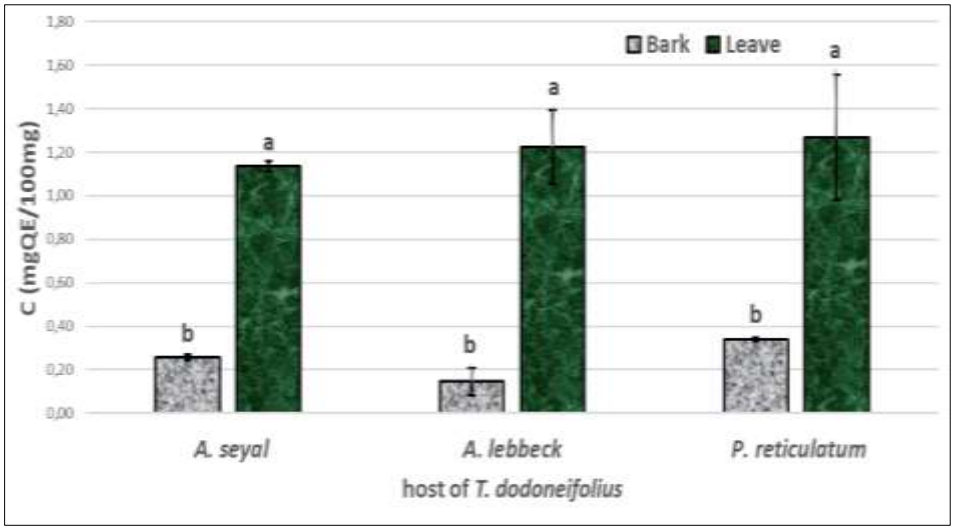

Fig 2: Total flavonoid content of methanolic extracts of leaves and bark of *T. dodoneifolius.* The results indicated by the distinct letters are statistically different (p< 0.05)

Antioxidant activity

The results of DPPH radical reduction activity by extracts are presented in figure 3. The assessment of free radical scavenging activity express in IC_{50} (µg/mL) showed 4.7±0.01; 2.61±0.16 and 2.40±0.24 respectively by bark extracts of *Tapinanthus dodoneifolius* harvested from *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum*. The leaf extracts of *T. dodoneifolius* showed IC_{50} (µg/mL) that are 3.86±0.11; 8.13±0.57 and 3.86±0.11 respectively by *T. dodoneifolius* harvested on *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum*. The IC_{50} value being inversely proportional to the anti-free radical activity, the extracts of *T. dodoneifolius* bark from *Piliostigma reticulatum* and *Albizia lebbeck* showed identical activities which are also the best compared to that of bark extract from *T. dodoneifolius* parasitizing *Acacia seyal*. At the same time the leaves of *T. dodoneifolius* from *Acacia seyal* and *Piliostigma reticulatum* have the same anti-radical activities and are higher than the leaves of *T. dodoneifolius* from *Albizia lebbeck*. Considering the organ used and the host, the barks of *T. dodoneifolius* from *Albizia lebbeck* and *Piliostigma reticulatum* showed higher antioxidant activities than the leaves. However, the opposite of this result was observed in *T. dodoneifolius* from *Acacia seyal*.

The IC_{50} values of reference compounds gallic acid and quercetin that we obtained are respectively 4.37µg/mL and 11.19µg/mL. The bark extracts of *Tapinanthus dodoneifolius* harvested from *A. lebbeck* and *P. reticulatum* showed higher activities than those of gallic acid. The correlation between leaves antioxidant activity and total phenolic content was significant (0.99) and moderate (0.56) to barks.

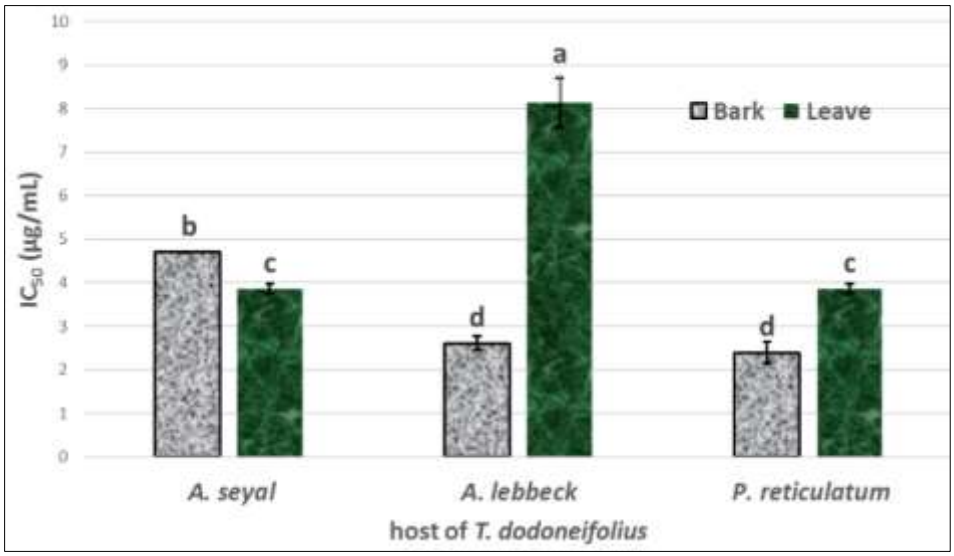

Fig 3: Antioxidant activity of leaves and bark of *T. dodoneifolius* harvested on *A. seyal*, *A. lebbeck* and *P. reticulatum*. The results indicated by the distinct letters are statistically different (p< 0.05)

IR analysis

The extracts IR spectra analysis allowed the identification of characteristic peaks of chemical groups. All extracts from the bark and leaves of *T. dodoneifolius* harvested on *A. seyal*, *A. lebbeck* and *P. reticulatum* show peaks around 1698cm^{-1} characteristic of the sesquiterpene lactone moiety (vibration of the O=C-C=C conjugated carbonyl bond).

The IR spectra of barks and leaves are respectively as follows, depending on the host:

A. seyal,1686 and 1697 cm^{-1}; *A. lebbeck*,1689 and 1698 cm^{-1}; *P. reticulatum*,1692 and 1717 cm^{-1}.

Peaks around 3351 cm^{-1} were recorded. They are characteristic of the hydroxyl group (OH) and are as follows: *A. seyal*, 3383 cm$^{-1;}$ *A. lebbeck*, 3299 and 3399 cm^{-1} and *P. reticulatum*, 3387 cm^{-1}.

UV spectrophotometric analysis

Samples analysis showed an absorbance maximum at 290 nm. This band indicates the presence of the α, β-unsaturated group of the γ-lactone ring.

All the analyses show that leaves extracts of *T. dodoneifolius* harvested from *A. lebbeck* and *P. reticulatum* contain sesquiterpenic lactones.

TLC profile of extracts

The result of sesquiterpene lactones identification by TLC method is shown by Figure 4. The chloroform-propan-2-ol-formic acid-water system (2: 8: 2:1 v/v) yielded the chromatogram shown in Figure 4. The developer with a low pH (pH=6) is specific for the detection of unsaturated sesquiterpene lactones. Spraying with hydroxylamine hydrochloride after heating to 105 °C, followed by spraying with ferric trichloride solution gives a brown-violet colouration. The brown-violet spots are observed in the chromatographic profile of *T. dodoneifolius* leaf extracts parasitizing *Piliostigma reticulatum* and *Albizia lebbeck*.

The appearance of the brown-violet colouration verifies the hypothesis of the presence of sesquiterpene lactones in the extracts of the leaves of *T. dodoneifolius* parasitizing *Albizia lebbeck* and *Piliostigma reticulatum*.

The extracts of *T. dodoneifolius* harvested from *P. reticulatum* (bark), *A. seyal* (bark and leaves) and *A. lebbeck* (leaves) did not contain sesquiterpenic lactones, or only in very small quantities.

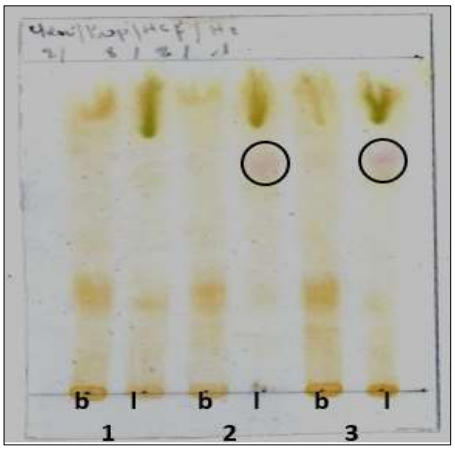

Fig 4: Chromatogram of extracts from the leaves and bark of *T. dodoneifolius*. The brown-violet spots are characteristic of sesquiterpenic lactones. Leaves of *T. dodoneifolius* harvested on *A. lebbeck* and *P. reticulatum* show brown-violet spots. 1(*A. seyal*), 2(*A. lebbeck*) and 3(*P. reticulatum*) are the hosts of *T. dodoneifolius*. b: bark; l:leaves

Discussion

This work consisted of a comparative study of leaves and twig bark from *Tapinanthus dodoneifolius* harvested on *Acacia seyal*, *Albizia lebbeck* and *Piliostigma reticulatum*.

The profiles and phytochemical contents of a plant can vary according to either the harvest period or its origin. These variations can also affect the biological properties of plants, on which the results of phytotherapy treatments directly depend.

The results obtained show that the twig barks of *T. dodoneifolius* would be better indicated for a possible extraction of phenolic compounds.

In contrast to the results found, Idu *et al*., [22] reported a variation in the content of secondary metabolites of *T. dodoneifolius* depending on its host. The phenolic contents of 18.55±0.21 and 18.33±0.45 mgEAG/100mg respectively of the macerate and decoctate of *T. dodoneifolius* harvested on *Vitellaria paradoxa* were reported [23].

Authors have reported flavonoid contents of 1.40±0.03 and 1.48±0.01 mgEQ /100mg respectively with a macerate and a decoctate of *T. dodoneifolius* harvested on *Vitellaria paradoxa* [23].

This difference can be explained by several parameters including the origin, the host of the plant and the extraction solvent used. The leaves would be better suitable for the isolation of flavonoids or for a treatment based on the biological activity of these compounds.

The metanolic extracts of *T. dodoneifolius* used in this work showed good antioxidant activities by scavenging the DPPH radical. Boly *et al*., [23] have reported 13.20±0.37 and 8.28±0.25 µg/ml as IC_{50} respectively with a macerate and a decoctate of *T. dodoneifolius* harvested on *Vitellaria paradoxa*. Ngom *et al*., [24] reported an IC_{50} value of 767.28±2.78 µg/mL with the methanolic extract of *T. dodoneifolius* parasitizing *Acacia albida*. Medicinal plants with antioxidant activities have been used for their properties to treat or prevent several human diseases in which oxidative stress appears to be one of the causes [25]. The use of *T. dodoneifolius* against pathologies associated with oxidative stress such as cardiovascular, allergic, convulsive and depressive diseases would be due to its antioxidant properties observed. It has been reported that the activity of a plant against cancer is related to its antioxidant properties which are dependent on its relative richness in polyphenol constituents [26]. The use of *T. dodoneifolius* against some cancers would be due in part to its antioxidant properties.

IR analysis of the extracts showed results that are similar to those of Ouedraogo *et al*. [27]. In UV analysis, Ouedraogo *et al*., [27] reported an absorption maximum at 275 nm. The shift would be due to bathochromic effect induced by the presence of hydroxyl and/or methoxyl groups.

It has been reported that sesquiterpenic lactones have shown properties such as neuroprotective, antiallergic, anticancer, anti-ulcer, anti-inflammatory, antidiabetic and bone remodelling [28]. Used sesquiterpenes have shown promising anti-inflammatory and anticancer activities by acting on various targets [29]. In addition, they exhibited antitrypanosomal, anxiolytic, antifungal and analgesic properties [30]. As this plant is used as fodder, it could be involved in the treatment of certain animal diseases [24] due to the presence of sesquiterpenic lactone. *T. dodoneifolius* is used against cancers [31] of the throat, larynx [32] and esophageal cancer [33]. It is used against allergies of the respiratory tract [6, 14]. The leaves are also used against infertility [3]. *T. dodoneifolius leaves* from *Tamarindus indica*

is used to treat ulcer [34]. Sesquiterpene lactones have shown properties against these ailments [35]. The use of the plant in treatments would be linked to the presence of its sesquiterpene lactones. *T. dodoneifolius* is used in the treatment of diabetes, and this property is linked to the presence of sesquiterpene lactones, which have shown such activity in other studies [32]. Sequiterpene lactones could be the source of *T. dodoneifolius's* medicinal capacity against epilepsy in phytotherapy [5]. The whole plante of *T. dodoneifolius* is used by Kaduna people in Nigeria to manage depression [36]. *T. dodoneifolius* twig bark was showed antidepressant activity [37]. As sequiterpene lactones are powerful anti-inflammatory agents, this would explain the use of *T. dodoneifolius* against inflammatory diseases of the nervous system, digestive tract, epidermis and joints [38]. Mamman *et al*., [7] reported no toxic effect of whole plant methanolic extract of *T. dodoneifolius*. *T. dodoneifolius*, through the diversification of its hosts, could be a source of investigation for the discovery of new bioactive molecules of therapeutic interest.

Conclusion

The study showed that the barks of *T. dodoneifolius* contain higher polyphenol contents than the leaves. Conversely, total flavonoid levels were higher in the leaves than in the twig barks. These high contents did not vary according to the host of the plant. The best antioxidant activity was presented by the twig barks of *T. dodoneifolus* harvested from *A. lebbeck* and *P. reticulatum*. Sesquiterpene lactones were identified in the leaves of *T. dodoneifolus* harvested from *A. lebbeck* and *P. reticulatum*. The variation in the presence of these secondary metabolites would justify the use of this plant according to its host in ethnomedicine.

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