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Research Article

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT EFFECT OF *SOLANUM ROSTRATUM* SPECIES FROM ALGERIA

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ABSTRACT

Objective: The aim of this study was to isolate the constituents and evaluate the antioxidant activity of *Solanum rostratum* Dunal (Solanaceae) from Algeria.

Methods: In this study, phytochemical analyses of the chloroform, ethyl acetate, and *n*-butanol extracts obtained from the aerial parts of *S. rostratum* were performed by column chromatography, thin-layer chromatography, and high-performance liquid chromatography techniques. The antioxidant activity was performed by 2,2-diphenyl-1-picrylhydrazyl scavenging assay method.

Results: The identification and structure elucidation of the isolated compounds were compared with their nuclear magnetic resonance spectra and the literature led to identify one monoterpene glycoside (linalyl-β-glucopyranoside) (1) and three flavonoid glycosides: Apigenin-7-*O*-glucoside (2), astragalin (3) and (isorhamnetin-3-*O*-glucoside (4).

Conclusion: This is the first report on the isolation and structure elucidation of compound (1) (linalyl-β-glucopyranoside) and compound (2) (apigenin-7-*O*-glucoside) from this species. In addition, antioxidant effect of the chloroform extract from *S. rostratum* was evaluated. The chloroform extract exhibited the remarkable radical scavenging ability (IC_{50}=0066±0.001 mg/mL).

Keywords: *Solanum rostratum*, Monoterpene glycoside, Flavonoid glycosides, Antioxidant.

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INTRODUCTION

Solanaceae family has an economical and medicinal importance [1], it comprising about 2000 species and growing in the tropical and sub-tropical areas [1]. A phytochemical investigation in this family showed several types of chemical constituents and many traditional uses. *Solanum torvum* Swartz used in folk medicine as sedative, digestive, hemostatic, and diuretic in the southern areas of China [2]. The main isolated constituents were flavonoids and saponins from the aerial parts [3], along with antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *S. torvum* [4]. The methanolic extract from the fruit of *Solanum lycocarpum* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats [5].

Solanum rostratum Dun is a harmful herb, it has widespread existence in North America [6] and has been classifying as quarantine weed for ecosystem and human health [7]. The previous isolation studies showed that *S. rostratum* had alkaloid components: Solasonine, solamargine [8], and methyl protodioscin [9], which cause the toxicity of this species [10]. In addition to exist the phenolic compounds which have various activities against antibacterial, cytotoxic and antioxidant [11,12].

In this study, we report the isolation and characterization of the constituents of *S. rostratum* and evaluation of antioxidant activity of the chloroform extract of this plant.

METHODS

Plant material

The aerial parts of *S. rostratum* were collected at the end of April 2009 (flowering stage) in Constantine, Algeria. Fresh aerial parts were dried to constant weight at room temperature.

Chemicals

Anhydrous sodium carbonate, Folin–Ciocalteu's phenol reagent, and methanol (analytical reagent and high-performance liquid chromatography [HPLC] gradient grade) were purchased from Merck (Darmstadt, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 2,6-di-*tert*-butyl-4-methylphenol (BHT), butylated hydroxyanisole (BHA), and dimethylsulphoxide (DMSO) were purchased from Sigma-Aldrich GmbH (Taufkirchen, Germany). All other chemicals were analytical grade and obtained from either Sigma or Merck.

General experimental procedure

Nuclear magnetic resonance (NMR) measurements were performed on a Bruker Avance III spectrometer in DMSO-d6 and CD_3OD (^{1}H: 600 MHz; ^{13}C: 150 MHz). Chemical shifts were given in ppm with tetramethylsilane as an internal standard. HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Shimadzu RID-10A refractive index detector and Shimadzu injector on a C18 l-Bondapak column (30 cm×7.8 mm, 10 µm waters, flow rate 2.0 ml min× 1). The absorbance measurements for antioxidant activity were recorded using the ultraviolet and visible spectrophotometer Rayleigh ultraviolet (UV)-2601, BRAIC Co. Ltd., Beijing, China.

Extraction and isolation

The aerial parts of *S. rostratum* (1430 g) were crushed and macerated 3 times with 80% aqueous methanol, each for 72 h at room temperature, and the solvent was removed *in vacuo* to yield a crude extract (269.2 g). The crude extract was dissolved in 800 ml H_2O and extracted with petroleum ether, chloroform, ethyl acetate, and *n*-butanol, respectively. The chloroform extract (2.1g) was subjected to column chromatography on silica gel using a $CHCl_3$–MeOH (from 100:0 to 0:100, v/v) to provide 26 fractions, and the fraction 13 (84.1 mg) was purified on preparative C18

HPLC column with a gradient of MeOH–H_2O (30:70, v/v) to yield compound (1) (3 mg). The ethyl acetate extract (5.44 g) was chromatographed on silica gel eluted with a gradient of petroleum ether–diethyl ether (from 100:0 to 0:100, v/v) to obtain 17 fractions, the fraction 9 (812 mg) performed on flash columneluted gradually with a $CHCl_3$–MeOH (from 100:0 to 0:100, v/v) to afford 13 subfractions, and the precipitate from subfraction 7 (128 mg) was purified by thin-layer chromatography (TLC) eluted with aEtOAc–MeOH–H_2O (8:1:1, v/v/v) to yield compounds 2 (9 mg) and 3 (11 mg). Fractionation of the n-butanol extract (17 g) was carried out by column chromatography on silica gel, eluted with a gradient of EtOAc–MeOH (from 100:0 to 0:100, v/v) to yield 21 fractions, and the yellow precipitate from fraction 17 (193 mg) was purified by TLC eluted with a EtOAc–MeOH–H_2O (8:1:1, v/v/v) to yield compound (4) (5 mg).

Antioxidant activity

DPPH radical scavenging assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH using the method of Blois (1958) [13], with some modifications [14]. The sample solutions were added to 0.004% methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank by a spectrophotometer (Rayleigh, UV-2601). Scavenging of DPPH radical was calculated according to the following formula:

$$\text{Radical scavenging \%} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. DPPH scavenging activity was expressed as IC_{50} values (mg/ml) for comparison. The IC_{50} value of each sample was defined as the concentration of sample required for a 50% decrease in absorbance of the blank. BHT and BHA were used as positive controls.

Determination of total phenolic contents

Total phenolic content of the chloroform extract was analyzed using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi [15] using gallic acid as standard, with some modifications [16]. The extract solution was mixed with 0.2 ml of 50% Folin–Ciocalteu reagent and allowed to react for 3 min, and 1 ml aqueous solution of 2% Na_2CO_3 was added. At the end of incubation for 45 min at room temperature, the absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid. Total phenolic content was expressed as μg gallic acid equivalents per mg of the extract.

Statistical analysis

All experiments were done in triplicate. The results were expressed as means±standard deviations. Statistical analyses were performed using the SPSS 11.5 (SPSS, Chicago, IL). Differences among means were done by analysis of variance, and averages were compared using the Tukey test. The level of statistical significance was taken at $p<0.01$.

RESULTS

The four compounds isolated from *S. rostratum* species which grow in Algeria were identified as linalyl-β-glucopyranoside (1) [17], apigenin-7-*O*-glucoside (2) [18], astragalin (3) [10], and isorhamnetin-3-*O*-glucoside (4) [19] (Fig. 1), by comparing their spectra data with those reported in the literature.

Compound 1 (Linalyl-β-glucopyranoside):

It is isolated as white viscous liquid. The ^{1}H NMR and heteronuclear single quantum correlation spectra of 1 (Figs. 2 and 3) exhibited signal attributed to one methyl at δ 1.39 (3H, s, H-10); two methyls at δ 1.61 (3H, s, H-8) and 1.69 (3H, s, H-9) which may connect with an olefinic carbon according to the downfield shift of the methyls; two olefinic methine signals with a proton at δ 5.99 (1H, dd, J = 17.5, 11.0 Hz, H-2) and 5.12 (1H, br t, J =7.0 Hz, H-6); two olefinic methylene signals with a proton at δ 5.22 (1H, d, J =11.0 Hz, H-1-a) and 5.27 (1H, d, J =17.5 Hz, H-1-b); and two methylene groups with signals at δ 1.61 (2H, m, H-4) and 2.06 (2H, m, H-5). One anomeric proton signal at δ 4.36 (d, J =7.5 Hz, H-1') corresponding to glucosyl moiety. One anomeric proton signal at δ 4.36 (d, J =7.5 Hz, H-1'), and five protons at δ 3.18 (m, H-2'), 3.29 (m, H-3'), 3.39 (m, H-4'), 3.19 (m, H-5'), 3.65 (dd, J= 11.8, 5.4 Hz, H-6'-b), and 3.81 (dd, J= 11.8, 2.2 Hz, H-6'-a) corresponding to glucosyl moiety. The sugar linkage and methyl group in position 10 were determined on the basis of the HMBC correlations of the quaternary carbon at δ 88.8 (C-3) (Figs. 4 and 5). ^{13}C NMR (MeOD, 150MHz): 16.0 (C-8), 21.9 (C-10), 22.7 (C-5), 24.0 (C-9), 40.0 (C-4), 61. 4 (C-6'), 70.2 (C-4'), 73. 0 (C-2'), 76.8 (C-5'), 77.0 (C-3'), 88.8 (C-3), 98.7 (C-1'), 114.4 (C-1), 124.4 (C-6), 130.6 (C-7), 142.5 (C-2).

Compound 2 (Apigenine-7-O-glucoside): yellow powder; $C_{21}H_{20}O_{10}$; ^{1}H-NMR (MeOD, 600MHz): 3.27~4.10 (6H, H-2''~H-6'', m), 5.78 (1H, d, J = 7.7 Hz, H-1''), 6.64 (1H, d, J = 2.0 Hz, H-8), 6.67 (1H, s, H-3), 6.82 (1H, d, J = 2.0 Hz, H-6), 6.94 (2H, d, J = 8.5 Hz, H-3', H-5'), 7.91 (2H, d, J = 8.5 Hz, H-2', H-6'); ^{13}C-NMR (MeOD, 150MHz): 61.65 (C-6''), 70.89 (C-4''), 74.34 (C-2''), 76.29 (C-3''), 77.90 (C-5''), 95.37 (C-8), 100.49 (C-6), 103.29 (C-3), 103.30 (C-1''), 109.22 (C-10), 116.22 (C-3', C-5'), 123.40 (C-1'), 128.20 (C-2',C-6'), 159.73 (C-4'), 160,25 (C-9), 162.10 (C-7), 163.85 (C-5), 164.20 (C-2), δ 181.53 (C-4).

Compound 3 (Astragalin): yellow powder; $C_{21}H_{20}O_{11}$; ^{1}H-NMR (DMSO-d6, 600MHz): 3.22~3.73 (6H, H-2''~H-6'', m), 5.42 (1H, d, J = 7.53 Hz, H-1''), 6.18 (1H, d, J = 2.01 Hz, H-6), 6.36 (1H, d, J = 2.01 Hz, H-8), 6.90 (2H, d, J = 8.78 Hz, H-5', H-3'), 7.08 (2H, dd, J = 8.78 Hz, H-6', H-2'); ^{13}C-NMR (DMSO-d6, 150MHz): 61.32 (C-6''), 69.95 (C-4''), 74.33 (C-2''), 76.68 (C-3''), 77.01 (C-5''), 96.76 (C-8), 99.28 (C-6), 102.83 (C-1''), 103.42 (C-10), 114.78 (C-3', 5'), 121.44 (C-1'), 130.84 (C-2', 6'), 133.97 (C-3), 157.28 (C-2),157.47 (C-9), 160.21 (C-4'), 163.35 (C-5), 165.89 (C-7), 178.03 (C-4).

Compound 4 (Isorhamnetin-3-O-glucoside): yellow powder; $C_{22}H_{22}O_{12}$; ^{1}H-NMR (DMSO-d6, 600MHz): 3.68~3.09 (6H, H-2''~H-6'', m), 3.83 (3H, s, 3'-OCH_3),5.42 (1H, d, J = 7.53 Hz, H-1''), 5.90 (1H, d, J = 2.26 Hz, H-6), 6.00 (1H, d, J = 2.26 Hz, H-8), 6.87 (1H, d, J = 8.78 Hz, H-5'), 7.98 (1H, dd, J = 8.78-2.50 Hz, H-6'), 8.00 (1H, d, J = 2.50 Hz, H-2'); ^{13}C-NMR (DMSO-d6, 150MHz): 56.88 (4'-OCH3), 72.93 (C-2''), 73.70 (C-3''), 69.93 (C-4''), 77.81 (C-5''), 60.91 (C-6''), 95.60 (C-8), 99.68 (C-6), 101.35 (C-1''), 103.80 (C-10), 113.50 (C-2'), 115.55 (C-5'), 121.59 (C-1'), 131.16 (C-6'), 133.50 (C-3), 147.59 (C-4'),149.99 (C-3'), 155.78 (C-2), 156.60 (C-9), 161.30 (C-5), 165.10 (C-7), 177.70 (C-4).

Antioxidant activity and total phenolic content

DPPH radical scavenging activities of the chloroform extract ($CHCl_3$) and the synthetic agents are given in Table 1. According to these IC_{50} values, the DPPH radical scavenging abilities among the samples were in the order of BHA>BHT>$CHCl_3$ ($p<0.01$). The IC_{50} value for DPPH radical scavenging of the chloroform extract was determined as 0.066±0.001 mg/ml. Fig. 6 shows the dose-response curves of the DPPH radical scavenging activities of the crude chloroform extract from *S. rostratum* and the synthetic antioxidants. On the other hand, the content of total phenolic compounds in the chloroform extract was found as 88.45±0.34 μg/mg.

Table 1: Antioxidant activities of chloroform extract from *Solanum rostratum*[1]

Material	DPPH	Total phenolic content
	IC_{50} (mg/ml)	(μgGAE/mg of extract)
Cloroform extract	0.066±0.001[c]	88.45±0.34
BHT	0.023±0.001[b]	NS
BHA	0.003±0.000[a]	NS

[1]Values represent averages±standard deviations for triplicate experiments. Values in the same column with different superscripts are significantly ($p<0.01$) different. NS: Not studied. DPPH: 2,2-diphenyl-1-picrylhydrazyl, BHT: 2,6-di-*tert*-butyl-4-methylphenol, BHA: Butylated hydroxyanisole

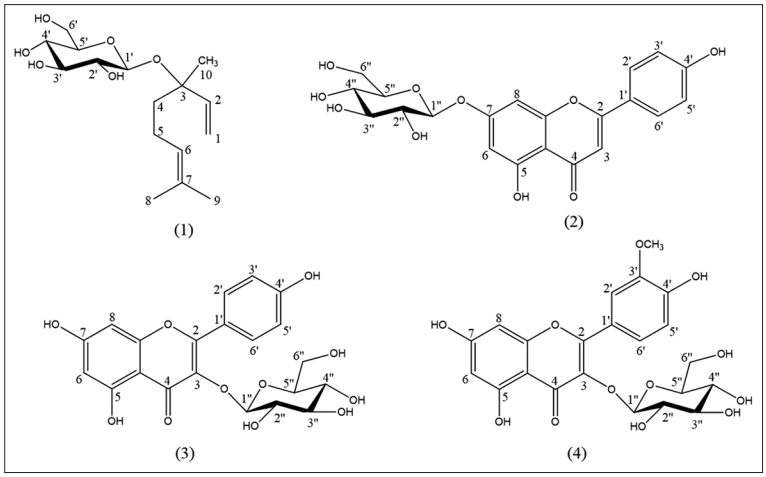

Fig. 1: Structures of the isolated compounds 1-4

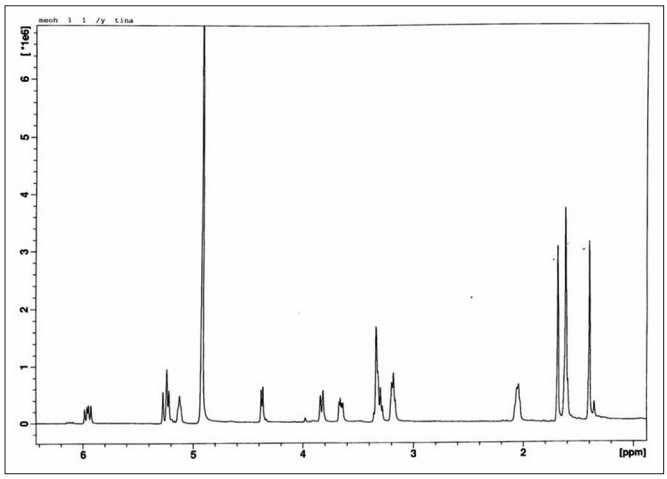

Fig. 2: ^{1}H-nuclear magnetic resonance spectrum of compound 1

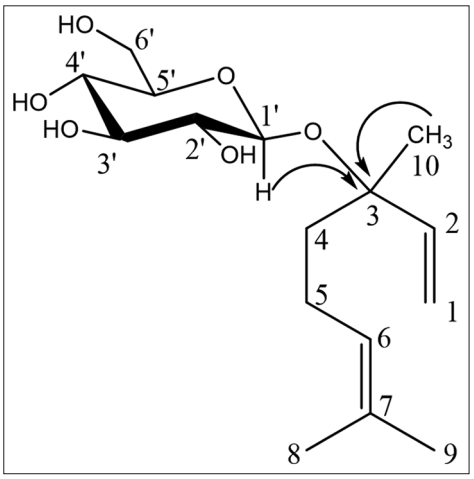

Fig. 4: Selected HMBC (→) correlations of compound (1)

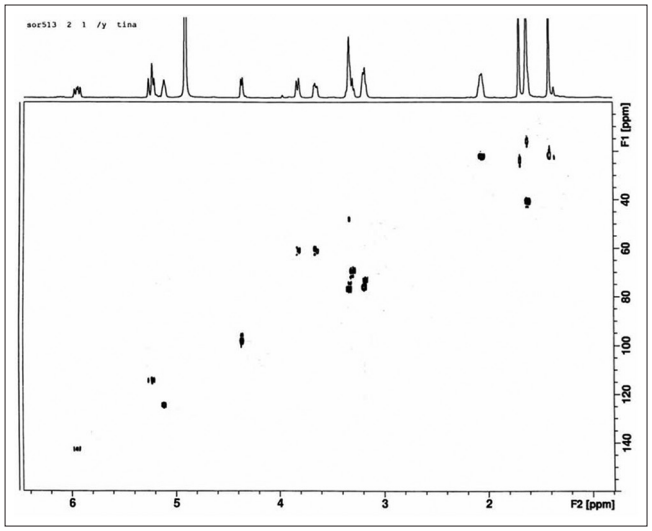

Fig. 3: Heteronuclear single quantum correlation spectrum of compound 1

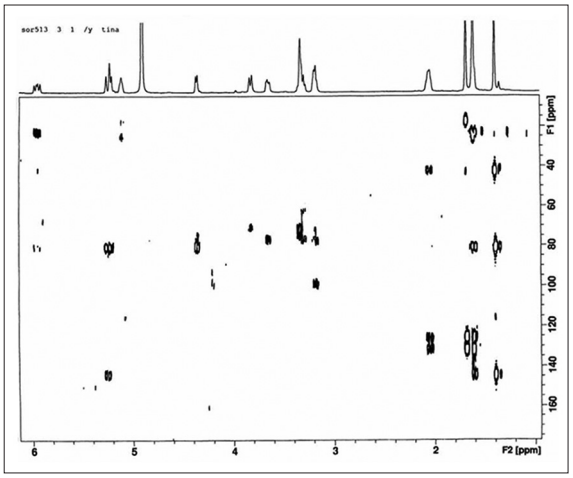

Fig. 5: HMBC Spectrum of Compound 1

DISCUSSION

Phytochemical analyses of the chloroform, ethyl acetate, and *n*-butanol extracts obtained from the aerial parts of *S. rostratum* led to the isolation of three flavonoid glycosides and monoterpene glycoside.

One of the aims of this study was to compare the constituents of *S. rostratum* species which grow in Algeria with the other species in different regions. Flavonoids and saponins are the most common isolated compounds which have been reported from the Chinese species *S. Rostratum* [20]. However, the phytochemical study of the Mexican medical plant *S. rostratum* Dun led to isolated one saponin (methyl protodioscin) [9]. *S. rostratum* species which grow in Azerbaidzhan

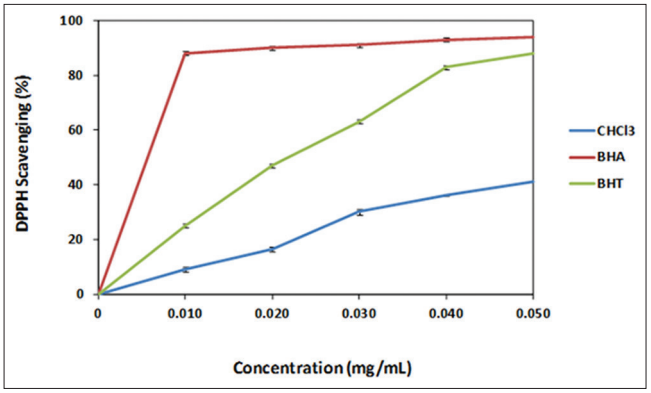

Fig. 6: 2,2-diphenyl-1-picrylhydrazyl scavenging activities of the samples at the tested concentrations

was collected in the Stepanakert region, and two glycosidic alkaloid compounds were obtained and identified as solasonine and solamargine.

In comparison to our results with the previous studies, it showed that the *S. rostratum* Dunal from China also produces compounds, isorhamnetin-3-*O*-glucoside and astragalin [10,19]. While compound (1) (Linalyl-β-glucopyranoside) is isolated for the first time from Algerian species S. rostratum and from the genus *Solanum.* In addition, this is the first report on the isolation and structure elucidation of compound (2) (Apigenine-7-*O*-glucoside) from this plant. The environmental factors are able to change or produce different secondary metabolites in the same plant species growing under different environmental conditions [21-27]. Thus, the deference of isolated compounds of *S. rostratum* species from different regions probably refers to the environment diversity, such as water availability, temperature, soil, and solar radiation [28-32].

Several studies have been reported on the antioxidant effects of different Solanum species [33,34]. Compound (1) (linalyl-β-glucopyranoside) was isolated from the chloroform extract which showed the significant antioxidant activity. Similar to our study, Hao *et al.* [35] reported that the different extraction fractions (ethyl acetate, n-butanol, petroleum ether, and water) of *S. rostratum* had strong antioxidant activities. In another study, the four methanolic extracts (roots, leaves, flowers, and fruits) from this species exhibited the significant inhibitions against the DPPH radical between 86.76 and 90.24% [36]. Since phenolic compounds are the most important groups of plant secondary metabolites that have diverse biological functions including antioxidant effects [37], the total phenolic content of the extract from *S. rostratum* was also determined. The results showed that chloroform extract of *S. rostratum* had a high amount of total phenolic content.

CONCLUSION

In this study, four compounds (linalyl-β-glucopyranoside, apigenin-7-*O*-glucoside, astragalin, and isorhamnetin-3-*O*-glucoside) were isolated from *S. rostratum* species which grow in Algeria. This is the first report on the isolation and structure elucidation of linalyl-β-glucopyranoside and apigenin-7-*O*-glucoside from this plant. Furthermore, in this study, the chloroform extract from this plant was evaluated for its antioxidant effect and the results showed that the chloroform extract was remarkable radical scavenger.

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AUTHOR'S CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflicts of interest.

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