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ESTUDIOS QUÍMICOS DE *AMBROSIA CUMANENSIS* KUNTH EN PANAMÁ

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RESUMEN

Ambrosia cumanesis K (Asteraceae), conocida como Altamisa, es una especie aromática distribuida ampliamente en el país. Se ha asociado su uso al tratamiento de afecciones renales, como antiinflamatorio, antiparasitario y purgante, por lo que se realizó la caracterización química de los extractos acuosos y etanólicos de las hojas de *A. cumanesis* K., así como de su aceite esencial, con el fin de evaluar su actividad biológica. Se llevaron a cabo pruebas de tamizaje fitoquímico para identificación de metabolitos secundarios, se emplearon métodos cromatográficos para la purificación de los extractos vegetales y la identificación de los compuestos principales se realizó por espectroscopía IR y de RMN, y por CG-MS para el caso del aceite esencial. El análisis químico de los extractos etanólicos de las hojas frescas de *A. cumanensis* Kunth llevó a la identificación de alcaloides, terpenos y flavonoides como los metabolitos secundarios mayoritarios. La extracción y purificación de los extractos llevó al aislamiento de tres (3) compuestos conocidos, psilostachina A, psilostachina

C y alcanfor. El análisis de la composición química del aceite esencial de esta planta ha producido la identificación de 25 compuestos, y mostró que la composición puede variar dependiendo de la estacionalidad. La actividad microbiológica de los extractos etanólicos y del aceite esencial fue evaluada por halo de inhibición, mostrando actividad antimicrobiana selectiva para el aceite esencial. Este es el primer reporte de estudios químicos sobre *Ambrosia cumanensis* Kunth en Panamá.

PALABRAS CLAVES

Ambrosia cumanensis K., aceite esencial, flora panameña, actividad biológica.

CHEMICAL STUDIES IN *AMBROSIA CUMANENSIS* KUNTH FROM PANAMA

ABSTRACT

Ambrosia cumanesis K (Asteraceae), known as Altamisa, is an aromatic species widely distributed throughout the country. Its use is associated with the treatment of kidney, anti-inflammatory, antiparasitic and laxative disorders. The chemical characterization of the aqueous and ethanolic extracts of the leaves of *A. cumanesis* K. was carried out, as well as of its essential oil, in order to evaluate its biological activity. Phytochemical screening tests were carried out to identify secondary metabolites, chromatographic methods were used for the purification of plant extracts and the identification of the main compounds was carried out by IR and NMR spectroscopy, and by CG-MS for the essential oil. The chemical analysis of the ethanolic extracts of the fresh leaves of *Ambrosia cumanensis* Kunth has led to the identification of alkaloids, terpenes and flavonoids as the major secondary metabolites. The extraction and purification of the extracts led to the isolation of three (3) known compounds, psilostachyn A, psilostachyn C and camphor. The analysis of the chemical composition of the essential oil of this plant has led to the identification of 25 compounds and the possible correlation between the composition variability and the seasonality. The microbiological activity of the ethanolic extracts and the essential oil was evaluated by inhibition halo, showing selective antimicrobial activity for the essential oil. This is the first report of chemical studies on *Ambrosia cumanensis* Kunth in Panama.

KEYWORDS

Ambrosia cumanensis K., essential oil, Panamanian flora, biological activity.

INTRODUCTION

The genus *Ambrosia* (Asteraceae) was described by C. Linneo (1753), includes around 45 species distributed in the Americas and has been known as a source of sesquiterpene lactones (Gupta, 1995). Until now more than 30 different related compounds are identified in *Ambrosia* species; ambrosanolides from *A. arborescens*; psilostachyins A-C from *A. tenuifolia* and pseudoguaianolides from *A. cumanensis* (Silva, 1992; Vera, 2008).

A. cumanensis is a perennial erect ragweed with simple or branched stem, white hairs mostly scattered. Opposite leaves at the base and alternates on the backs. Flowers yellowish-green colored with very short simple hairs (Gupta, 1995).

The chemical composition of the essential oil extracted from the fresh leaves of *A. cumanensis* Kunth has been reported in two previous studies. Payne *et al.* (1976) identified caryophyllene (20.4-36.2%), humulene (4.0-11.7%), terpinene (0.1-7.8%) and a mix of farnesenes (2.7-7.3%) as the major compounds and 24 additional compounds, mostly of them not identified. Years later, Ciccio & Chaverri (2015) reported a total of 137 compounds (about 90% of the total amount of the oil) isolated from a plant in Costa Rica, being bicyclogermacrene (14.7-23.4%), germacrene-D (10.1-16.9%), α-pinene (7.8-12.8%), β-pinene (4.5-6.7%) and chrysanthenone (6.2-8.7%), the most abundant compounds.

The phytochemical screening of the ethanolic extract of the leaves identified the presence of alkaloids, cardiotonic glycosides, quinones, flavonoids, tannins, carbohydrates and saponins. Bolhmann (1977) made the first reports of sesquiterpenes, altamisin, ambrosin and 2,3-epoxy ambrosin, isolated from the polar extract. Borges *et al.* (1983) described two new compounds: psilostachyins and isopaulitin obtained from the methanolic extract of the aerial parts. Cumambrins A-B and cumanin, guaianolides and pseudoguaianolides respectively, have been isolated from the ethanolic extract of a mexican specimen (Romo *et al.*, 1966). Aponte *et al* (2010) reported the identification of confertin and damsin, two pseudoguaianolides which exhibited significant

activity against a panel of human tumor cell lines. Other studies reported the ethanolic extract also showed antibacterial and antifungal activity (Lentz, 1998; Mesa *et al.*, 2017).

In Panamá, *A. cumanensis* is found in Darien, Colón, Chiriquí and Panama City. It is used in the ethnobotanical medicine as spasmodic, for the headache treatment, gastric disorders and depurative. The leaves are used to treat rheumatism; while an infusion of them is used to induce or reduce menstruation. Beyond those traditional uses, there is no scientific report about the chemical composition and biological activity of the secondary metabolites present in this specie in Panama (Gupta *et al*., 2000; Correa *et al*., 2004).

MATERIALS AND METHODS

Plant Material

The aerial parts of *A. cumanensis* K. were collected from San Pablo, David, Chiriqui Province, Panama, during the summer of 2016 at an altitude of 100 msnm and was identified by Prof. Rafael Rincón. The voucher specimen (N°C-020-10-2015) was deposited in the Universidad Autónoma de Chiriquí Herbarium.

Extraction and isolation of *A. cumanensis* crude extracts

Fresh aerial parts of *A. cumanensis* K. (100.1 g) were cut, divided in two portions and extracted two times with ethanol (96% v/v) and water at room temperature for 7 days, respectively. Subsequently the extracts were stored in a freezer at -15° C until further analysis. A third extract was obtained from the ethanolic treatment of dried leaves (21.5 g) and submitted to dynamic maceration for 7 days at room temperature.

All the extracts were analyzed by phytochemical screening to determinate its secondary metabolite composition. The ethanolic fresh leaves extract was centrifuged and concentrated under reduce pressure at 38-40° C and passed through an acid base extraction; the aqueous phase was extracted three times with EtOAc, to yield an EtOAc soluble fraction (8.0g). This organic fraction was subjected to silica gel column chromatography (100-250 mesh) with a

Ligroin/$CHCl_3$/Acetone (3:1:1) elution to give seven (7) fractions. Five of these fractions (F1-F5) were concentrated under reduce pressure and analyzed by IR and NMR spectroscopy.

Essential Oils of *A. cumanensis* K.

For the essential oil extraction, 200 g of fresh leave material and 2 liters of distilled water were distillated in a hydrodistillation system at atmospheric pressure for four hours. The distillated oils were collected in dry and wet season, extracted with chloroform, dried over anhydrous sodium sulfate, filtered and stored at 0° C until further analysis. A second methodology, an infusion preparation, was done to compare the better oil extraction method.

Gas Chromatography

The analyses by gas chromatography coupled to mass selective detector were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST computer databases. The data were obtained on a 5% phenyl-95% dimethylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μm), (MDN-5S). Operating conditions were, carrier gas He, flow 1.4 mL/min; oven temperature program: 60-280 °C at 3 °C/min; sample injection port temperature 250 °C; detector temperature 250 °C; ionization voltage: 70 eV; ionization current 60 μA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

Compound Identification

The components of the oils were identified using the retention indices which were calculated in relation to a homologous series of n-alkanes, on a 5% phenyl-95% dimethylpolysiloxane type column and by comparison of their mass spectra, the Kovacs indexes and the CIPRONA database. To obtain the retention indices for each peak 0.8 μL of the essential oils mixture was injected under the same experimental conditions reported above. Integration of the total chromatogram (GCFID) expressed as area percent and has been used to obtain semi-quantitative compositional data.

Microbiological test

Agar inhibition area test (Mueller-Hinton): The altamisa extracts were dissolved in water to obtain 0.5, 50 and 100% sample solutions. A volume of 1 µL of each solution was placed onto sterile filter disks and allowed to dry at room temperature. Each disk was placed on the surface of Mueller-Hinton medium agar which has been previously inoculated with standardized inoculum suspension of *Escherichia coli* (Escherich, 1885), *Staphylococcus aureus* (Rosenbach, 1884), *Pseudomonas aeruginosa* (Schroeter, 1872) *Citrobacter spp*.(Werkman and Gillen, 1932) *y Klebsiella sp.*(Trevisan, 1885) at 37° C for 24 hours. All the extracts were compared against positive control (AMP 10 µg, CIP 5 µg). Test were performed in triplicate.

RESULTS & DISCUSSION

The phytochemical analysis of all the extracts showed significant differences between the aqueous and ethanolic extracts of the aerial parts, ethanolic extracts being the richest one. Meanwhile, no significative differences were found between fresh and dry leaves ethanolic extracts. In both cases, alkaloids, tannins, terpenoids and steroids were detected; sesquiterpene lactones were present in the dry leaves either. The results are summarized in Table 1.

Fraction 3 (6.7 mg) was a brownish liquid that correspond to psilostachyin C, identical in all aspects (IR and NMR spectrum) with the psilostachyin C isolated from *A. artemisiifolia* (Stefanovic *et al*. 1972). Fraction 4 (20.2 mg) was a dark oil liquid that correspond to psilostachyin A, identified by NMR comparison of literature data. Additionally, a white solid (34.6 mg), identified as camphor was isolated from the extract.

From the hydrodistilled oil analysis by GC-MS, a total of twenty-five (25) compounds were identified, counting for the 74.57 % of the total chemical composition. Table 2 showed the identified components in order of elution from the MDN-5S column, the relative percentage of each one and the experimental retention time.

Table 1. Phytochemical screening results of the ethanolic and aqueous extract of *Ambrosia cumanensis* K.

TEST	METABOLITE	FLAE		FLEEx		DLEEx	
		RESULT	COLOR	RESULT	COLOR	RESULT	COLOR
DRAGENDORFF		+	Brownish precipitate	+++	Brownish precipitate	+++	Brownish precipitate
MAYER		++	Brownish precipitate	++	Brownish precipitate	+++	Green precipitate
WAGNER	**ALKALOIDS**	+++	Brownish precipitate	+++	Brownish precipitate	++	Brownish precipitate
HAGER		+	White precipitate	+++	Precipitado cristalino	+++	Brownish precipitate
WATER	**SAPONINS**	+++	Foam formation	-----	Foam formation (30 sec)	----	Foam formation
ROSENTHALER		----	Brown-greenish color	-----	Green color	+++	Violet color
FERRIC CHLORIDE ($FeCl_3$)	**TANNINS**	++	Brown-greenish color	+++	Brown-greenish precipitate	+++	Brown-greenish color
GELATINA-SAL		++	Brown-greenish color	+++	Green color	+++	Brown-greenish color
SHINODA		----	Brown-greenish color	+	Brown color	----	Brown-greenish color
NaOH	**FLAVONOIDS**	----	Brown-greenish color	----	Green color	+++	Coloración amarilla
LIEBERMAN-BUCHARD	**TERPENS AND STEROIDS**	++	Yellow interfase	+++	Yellow interfase	+++	Blue interfase
SALKOWSKI		++	Yellow interfase	+++	Coloración verde en la interfase	+++	Blue interfase
KOH 10%	**CUMARINS**	----	Brown-greenish color	+	Fluorescence	----	----
BALJET		----	Green color	----	Green color	+++	Orange-red color
LEGAL	**SESQUITERPPENE LACTONES**	----	Green color	----	Green color	----	Yellow color

FLAE: fresh leaves aqueous extract, FLEEx: fresh leaves ethanolic extract, DLEEx: dried leaves ethanolic extract.

(+++) strong intensity reaction, (++) médium intensity reaction, (+) weak intensity reaction (--) not detected.

Table 2. Chemical and percentage composition of *Ambrosia cumanensis* Kunth essential oil from Panama

n°	Compounds	Retention time	relative amount (%)	Retention Index*	Class**	Method
1	Acetic acid, 2-propenyl ester	3.041	9.4	676	A	Infusion
2	Pentanal, 2,2-dimethyl-	4.992	1.59	821	A	Infusion
3	3-Decanone	6.202	0.4	1151	A	Infusion
4	1-Hepten-6-one, 2-methyl-	7.661	1.24	920	A	Infusion
5	1,6-Octadien-3-ol, 3,7-dimethyl-	10.337	1.5	1082	A	Infusion
6	2-Cyclohexen-1-one, 4-ethylidene-3,5-dimethyl-	11.09	5.81	--	A	Infusion
7	β-Citronellene	12.113	5.71	1132	M	Infusion
8	1,4-Pentadiene, 2,3,3-trimethyl-	13.446	7.96	689	A	Infusion
9	Cyclopropanemethanol	13.599	2.97	664	A	hydrodistillation
10	Terpinen-4-ol	13.603	2.77	1137	M	Infusion
11	α-Terpineol	14.335	2.18	1143	M	Infusion
12	3-Cyclohexene-1-methanol,	14.367	2.28	1005	A	hydrodistillation
13	(E/Z)-2,5-dimethyl-1,3-hexadiene	14.813	3.98	728	A	Infusion
14	(*R*)-Carvone	17.489 - 17.610	2.36- 9.28	1190	M	Infusion
15	3,5-Heptadien-2-ol, 2,6-dimethyl-	19.665	2.9	1001	A	hydrodistillation
16	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	23.684	2.66	1140	A	Infusion
17	trans-Caryophyllene	26.373- 26.41	9.48- 14.47	1418	S	hydrodistillation
18	α - humulene	26.909- 26.962	0.64- 3.48	1449	S	hydrodistillation
19	Methyl (*E*)-2-(3-cyclopropyl-7-norcaranyl)	30.564	9.4	1431	A	hydrodistillation
20	Longipinene epoxide	32.179-32.190	1.58- 2.27	1293	M	hydrodistillation
21	Dihydrocarvyl acetate	36.401	20.35	1335	M	hydrodistillation
22	3-Hexadecyloxycarbonyl	43.101	1.73	--	A	hydrodistillation
23	Oxalic acid	43.102	1.09	933	A	hydrodistillation
24	Valeric acid	49.09	1	875	A	hydrodistillation
25	nonadecanal	49.495- 49.522	6.41- 14.19	2100	A	hydrodistillation

* Expressed as Kovats Retention Index
**Class: A=aliphatic, M=monoterpene, S=sesquiterpene.
Hydrodestillation time: 3 hours (100 g fresh material/1.5 L distilled water)
Infusion time: 1 hour (100 g fresh material/1.0 L distilled water)

Predominantly, the altamisa essential oils were terpenoid in nature. In the dry season hydrodistillation sample, *R*-carvone (9.28%) and nonadecanal (6.41%) were the major compounds. In the rainy season hydrodistillation sample, dihydrocarvyl acetate (20.35%), nonadecanal (14.19%) and *trans*-caryophyllene (9.48%) were the main identified components, meanwhile the infusion showed *trans*-caryophyllene (14.47%), acetic acid, 2-propenyl ester (9.40%) and 1,4-pentadiene, 2,3,3-trimethyl (7.96%) as major compounds. (Figure 1).

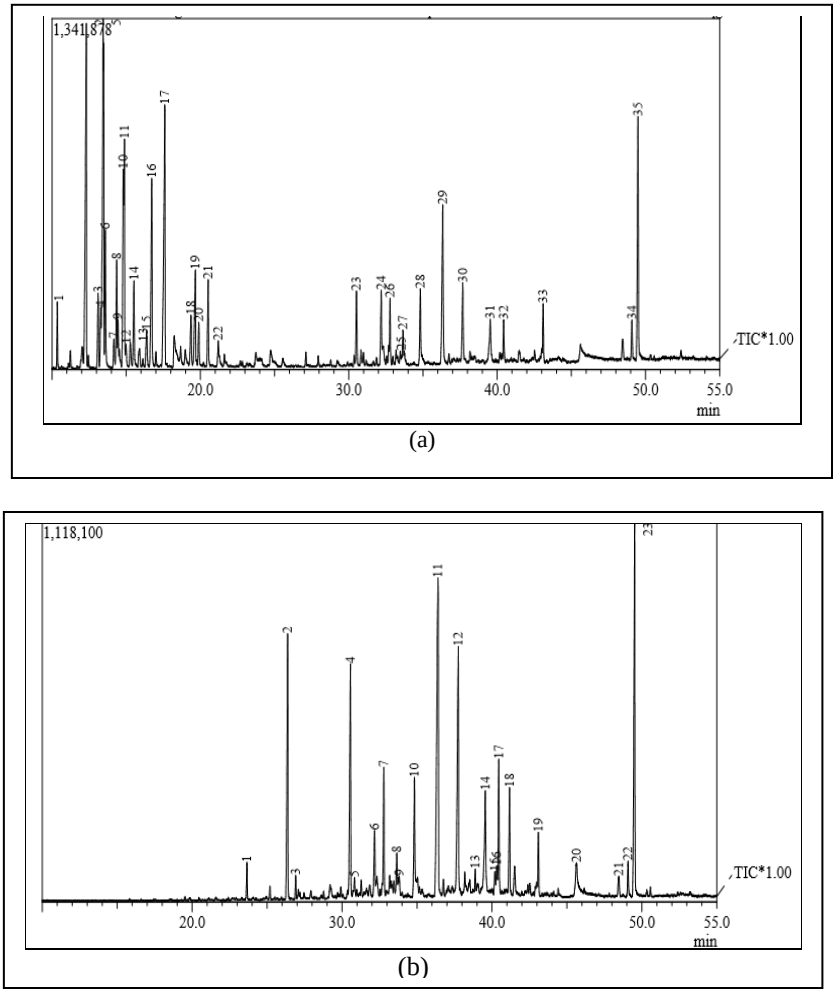
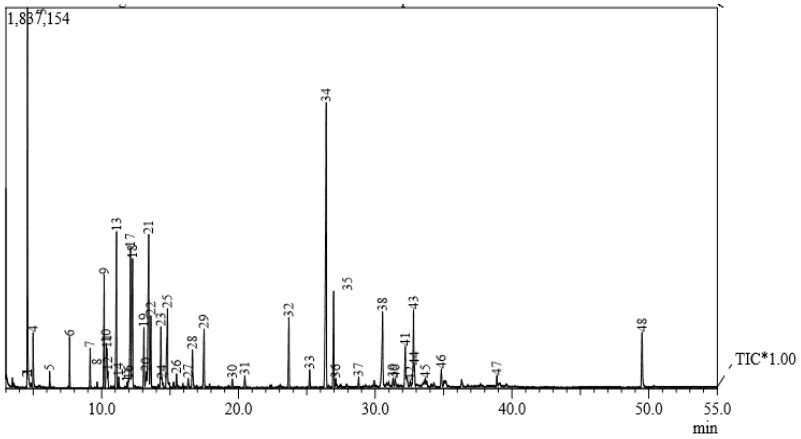


Figure 1. GC-MS spectrum: a) Dry season hydrodistillation sample, b) Rainy season hydrodistillation sample,



(c)

Continuation of figure 1: c) Infusion sample

The analysis of the literature related to several species of *Ambrosia* showed that this genus is rich in terpenoids, especially mono and sesquiterpenes. It is well known that the chemical composition in *Ambrosia*, as in other Asteraceae plants depends mostly of the environmental and grow conditions (Miller *et al*., 1968; Rodríguez *et al*., 1976). In Panamá, October is the rainiest month and July is a transition month between dry and rainy season. Our data revealed that exist significative differences between the season and method of extraction in accordance with these previous reports.

The major components detected in our study were aliphatics (17), with nonadecanal (6.41-14.19%) and acetic acid, 2-propenyl ester (9.40%) as major components; monoterpenoids, like *R*-carvone (2.36- 9.28%) and its acetate dehydrated derivate (20.35%). Sesquiterpenoids were the less abundant compounds with *trans*-caryophyllene (9.48- 14.47%) and α - humulene (0.64- 3.48%) as the only identified components. From these six compounds just the *trans*-caryophyllene has been previously reported in some *Ambrosia* genus.

Most reports identified monoterpenes and sesquiterpenes as the most abundant components in the *Ambrosia* oil, but our results showed

significative differences, as aliphatic compounds, especially lineal aldehydes, are the principal ones (Wang *et al*., 2006; Sulsen *et al*. 2008). Probably the phenology of the plant is a primordial factor in this behavior. *Ambrosia* is a complex genus affected by environmental aspects, so it is not surprising the variability founded.

The extracts tested by microbiological activity showed no selectivity between the different bacteria lines. For example, the essential oil extract was active against gram positive and gram negative bacterial lines, except for *E. coli*. Other studies have evaluated the antibacterial activity of the essential oil of *A. peruviana* by the diffusion method in agar, obtaining activity against *S. aureus*, *E. faecalis*, *E. coli* and *Salmonella typhi* (Eberth) Schroeter, with MIC values of 350-500 μg / mL (Yánez et al., 2011).

Meanwhile, the crude ethanolic extract showed activity only against some gram negative bacteria (*E. coli* and *P. aeruginosa*); Guauque *et al*., have evaluated the antibacterial activity of the ethanolic extracts of the plant on Gram positive bacteria *S. aureus* and *Streptococcus pyogenes* Rosenbach, and Gram negative *P. aeruginosa* (Schroeter) Migula, *E. cloacae, P. vulgaris, E. coli* Escherich and *E. coli* DH5α, which no presented activity. Camphor was inactive for all the bacteria lines. (see Table 4).

Table 3. The chemical class distribution in the essential oil of *Ambrosia cumanensis* Kunth from Panama

Chemical class	Fresh leaves
Aliphatics (A)	25.73
Alcohols	9.65
Aldehydes	22.39
Ketones	7.45
Esters	9.4
Total	**74.62**
Terpenoids	
Monoterpenoids (M)	46.5
Monoterpene hydrocarbons	---
Oxygenated monoterpenes	---
Sesquiterpenoids (S)	28.07
Sesquiterpene hydrocarbons	---
Oxygenated sesquiterpenes	---
Total	**74.57**

Table 4. Microbiological activity results (Inhibition zone in mm).

Code	Sample	Klebsiella spp	Escherichia coli	Citrobacter spp	Pseudomonas aeruginosa	Staphylococcus aureus
1	M1a	-	-	-	-	-
2	M1b	-	0.87 cm	-	0.83 cm	-
3	M2a	-	-	-	-	-
4	M2b	-	1.0 cm	0.90 cm	-	-
5	M3a	-	-	-	-	-
6	M3b	-	-	-	-	-
7	M4a	-	-	0.83 cm	0.77 cm	0.80 cm
8	M4b	0.77 cm	-	0.77 cm	0.80 cm	0.77 cm

M1: crude ethanolic extract I, M2: crude ethanolic extract II, M3: camphor, M4: Altamisa essential oil extract.

CONCLUSIONS

The analysis of the aerial parts of *Ambrosia cumanensis* K. showed the presence of alkaloids, lactone sesquiterpenes and phenolic compounds in the phytochemical screening. The major components detected in the essential oil were aliphatic compounds and monoterpenoids, sesquiterpenoids were the less abundant compounds. Meanwhile, Psilostachyin A, C and camphor were isolated from the ethanolic leaves extracts of the plant. The differences in the antibacterial activity of the essential oil and the ethanolic extracts corroborate the variability in the chemical composition of the genus *Ambrosia*, which depends mainly on environmental aspects and the extraction techniques used, as well as on the type of bacterial strain.

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