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Essential oil Composition, Antioxidant and Antibacterial Activities of *Jatropha tanjorensis* (*Euphorbiaceae*)

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Abstract

Background: *Jatropha tanjorensis* (*Euphorbiaceae*) leaves are traditionally used for the treatment of diabetes and also, possess antibacterial potential. The chemical composition, antioxidant and antibacterial activities of the oil were investigated. The essential oil (EO) was obtained by hydro-distillation method and characterised using gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was investigated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging technique and antibacterial activity of the volatile oil was evaluated against 6 strains of bacteria: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis and Salmonella typhi, using broth dilution method.

Results: The EO produced a percentage yield of 0.3% (w/w) and a total of 48 compounds were identified in *J. tanjorensis* leaf EO accounting for 75.16% of the total oil composition. The dominant compounds identified in the EO were phytol (8.92%), n-Nonacosane (7.35%), and β-Cis-Ocimene (7.29%). The EO showed a potent antioxidant activity when compared to the Butylated hydroxyanisole used as a standard drug, with % inhibition varying from 93.21 ± 0.02 to 92.90 ± 0.01 at 1000-125 µg/mL. The leaf essential oil exhibited good antibacterial activity on all the tested organisms at 1000-125 µg/mL.

Conclusion: The chemical composition and biological properties of *J. tanjorensis* leaf EO was determined for the first time and findings support the rationale for its medicinal applications.

Keywords: *Jatropha tanjorensis*; Antioxidant; Antibacterial activity; Gas chromatography-Mass spectrometry; Essential oil

Abbreviations: EO: Essential oil; GC-MS: Gas chromatography-mass spectrometry; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; J. tanjorensis: Jatropha tanjorensis; RT: Retention time; RI: Kovats retention index; JTE: Leaf essential oil of Jatropha tanjorensis; BHA: Butylated hydroxyanisole; ZOI: zone of inhibitory

Background

A medicinal plant chemical constituent contains substances that have therapeutic effects, or which are precursors for the synthesis of useful drugs. Various parts of plants such as the leaf, stem, bark, root, etc. are being used to prevent and cure various

ailments like malaria, diarrhea, tuberculosis, pneumonia, and asthma. Most of the potent medicinal plants have relatively no toxic or adverse effects when used by humans, their usage presently is on the increase due to easy availability, affordability, accessibility, and promising efficacy comparable to the often-high cost of the standard synthetic drugs Bakkali [1]; Bruneton [2] *Jatropha tanjorensis* belongs to the *Euphorbiaceae* family. It is a gregarious shrub and commonly called "Hospital too far" in Nigeria to portray its medicinal potential. *J. tanjorensis* leaf is edible and serves as a food in southern Nigeria. The leaf extracts have been reported to be used in the treatment of diabetes or hypoglycaemia Olayiwola [3]. All parts of the plant including seeds, leaves, and fresh stem bark or decoction of the plants' parts are used in traditional and folk medicine. The leaves of *J. tanjorensis* also have been reported to have the potential for the treatment of cardiovascular diseases Oyewole & Akingbala [4]. The fresh leaves of *J. tanjorensis* are reported to possess blood-replenishing properties and are used in treating blisters, dry skin, and bruises. It is also having an antibacterial activity Iwalewa [5]; Arsari [6]. In continuation of our search for new potent antioxidants and antibacterial from plants with ethno-medicinal reported uses Odeja [7]; Odeja [8]; Okpala [9]; Okpala [10], we report the chemical composition, antioxidant and antibacterial activities of leaves essential oil of *J. tanjorensis* which has not been reported in the literature.

Methods

Plant material

The fresh leaves of J. tanjorensis were collected from the Nsukka, Enugu State of Nigeria (6o51' 39''N; 7o 24'21''E). The plant was identified by Dr. S. K. Odewo of the Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria.

Isolation of essential oil

The fresh leaves were air-dried and pulverized. The essential oil was extracted using the Hydro distillation method with all glass Clevenger-type apparatus for 3 hours according to the British Pharmacopoeia specifications (British Pharmacopoeia [11]). The percentage yields (w/w) of volatile oils obtained were calculated and the oil was preserved at 4°C using a refrigerator before being subjected to GC-MS analysis and biological assay.

Gas chromatography-mass spectrometry of the volatile oil

The essential oil was subjected to GC-MS analysis on an Agilent 7809A Gas Chromatography hyphenated with an Agilent mass detector having split/split less injector interfaced with a mass selective detector operating at 70 eV. The ion source temperature was set to 200°C over a mass spectral range of m/z 50–700 at a scan rate of 1428 amu/sec. The column of the GC used was HP-5MS with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25µm. The oven temperature was programmed at an initial temperature of 80 °C for 2 min, increased at 10 °C/min to the final temperature of 240 °C for 6 mins. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection volume, linear velocity, and pressure were adjusted at 1.0µl, 362 cm/s, and 56.2 KPa, respectively. The relative percentages of the essential oil components were obtained by FID peak-area normalization. Identification of the essential oil components was based on a comparison of their mass spectral fragmentation patterns with a pre-installed NIST MS search 2.0 database NIST data and with the data previously reported data in the literature.

Antioxidant assay

The antioxidant investigation of the *J. tanjorensis* leaves essential oil was studied using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability method, with some modifications Brand-Williams [12]. Five concentrations of the volatile oils and standard antioxidant (Butylated hydroxyl anisole) (1000-125) µg/mL were prepared. 2.0 mL of 100 µM methanolic DPPH solution (used as control) was added to the five concentrations of volatile oils and incubated at room temperature for 30 minutes. The absorbance of the treated essential oil samples at different concentrations and the blank 100 µM methanolic DPPH solution (control) were then measured at 517 nm with a GS UV-12, UV-V is spectrophotometer. The analyses were carried out in triplicates and the average values were obtained. The activities of all the analysed samples were calculated as a function of their % inhibition using the equation

$$\%\text{Inhibition}\,\frac{ABc - Abs}{ABc}x100$$

Where ABc = absorbance of control; Abs = absorbance of samples

Antibacterial assay

Test organisms

Six strains of bacteria; four Gram-negative: *Escherichia coli, Salmonella typhi, Klebsiella pneumonia, and Pseudomonas aeruginosa and two Gram-positive: Staphylococcus aureus and Bacillus subtilis* were used in this study. All bacteria microbes were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria.

Preparation of Sample Solution

Oil samples were prepared such that 1 mL of the oil was regarded as 1000 µg/mL; 0.5 mL of this essential oil was taken into 0.5 mL of methanol to give 500 µg/mL. More serial dilutions gave different concentrations such as 250 µg/mL, 125 µg/mL and 62.50 µg/mL. The 6th and 7th test tubes were negative control (DMSO) and positive controls (Gentamycin) respectively [13].

Antibacterial Assay – Broth Dilution Method

Overnight cultures of microorganisms were prepared by taking two loopful of already grown organisms from the stock and inoculating each into the sterile nutrient broth of 5 mL each for 18-24 hrs at 37 ºC. The inoculation of six strains, from standardised suspensions, was made using a Sterr multi-

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inoculator using suspensions standardised at 0.5 MacFarland, with a second dilution performed onto BHI to obtain inoculum of an approximate concentration of 10^5–10^6 CFU/mL according to the method described by [14]. From the overnight culture, 0.1 mL of each organism was added to 9.9 mL of sterile saline (0.85 %) to get 10^{-2} dilutions of each organism. From the diluted organism, 0.2 mL was taken into the prepared sterile nutrient agar, which was at 45 ºC. This was aseptically poured into the concentrations of each essential oil and the control. The plates were incubated for 18-24 hrs at 37 ºC. Each experiment was carried in duplicates and the antimicrobial activity was evaluated by measuring the diameter of the inhibition growth zone in millimeters (mm) for the test organisms compared with Gentamicin (10 µg/mL) which was used as a positive control.

Statistical Analysis

All data represent the means of three replicates ± standard deviation. Results were subjected to analysis of variance (ANOVA) and the mean comparisons were performed by Bonferroni's Multiple Comparison Test using the statistical analysis software package (GraphPadPrism 5).

Results

Fresh leaves of *J. tanjorensis* (300 g) were used in hydrodistillation extraction to produce 0.9 g of colourless volatile oil with a yield of 0.3% (w/w) (Table 1). The GC-MS chromatogram of the essential oil is given in Figure 1. The chemical compositions of the essential oil were presented in Table 2. Forty-eight compounds were identified from leaf essential oil of *J. tanjorensis* accounting for 75.16% of the total composition of the oil. The classes of compounds identified in the oil are sesquiterpenes and oxygenated sesquiterpenes (31.63%), n-alkanes (13.73%), diterpene (8.92%), monoterpene (7.29%), alkanoic acids (4.02%), esters (3.87%), alkanols (2.37%), alkynes (2.06%) and alkenes (1.27%) (Table 2). The DPPH radical scavenging activity of the essential oil of the leaves of *J. tanjorensis* is presented in Table 3, and the antibacterial activity of the volatile oil was determined based on inhibition zones observed against the four gram-negative and two gram-positive bacteria strain at various concentrations (1000-62.5 µg/mL) as compared to Gentamycin (standard antibacterial drug) (Table 4) & (Figure 2).

Table 1: Yields and properties of the leaf essential oil of *J. tanjorensis*.

S/N	Name	Weight (g)	Weight of Oil (g)	Approximate % Yield of oil	Physical properties
1	*J. Tanjorensis*	300	0.9	0.3	Colourless, herbal smell

Table 2: Chemical Composition of Essential oil from leaf essential oil of *J. tanjorensis*.

No	RT (mins)	Compounds	Class of Compound	Molecular Formula	RI [a]	RI [b]	% Composition
1	3.270	3-Nonanol	Alkanol	$C_9H_{20}O$	676	1090	0.51
2	3.384	2-Octanol	Alkanol	$C_7H_{18}O$	774	997	0.56
3	3.541	1-Methylcyclopentanol	Alkanol	$C_6H_{12}O$	781	796	0.29
4	3.962	n-Decane	Alkane	$C_{10}H_{22}$	787	160	0.25
5	4.045	Trans-3-Hexenyl acetate	Ester	$C_8H_{14}O$	1000	1004	1.04
6	4.468	β-Cis-Ocimene	Monoterpene	$C_{10}H_{16}$	496	1040	7.29
7	5.376	Cis-2-Nonenol	Alkanol	$C_9H_{18}O$	865	1120	1.01
8	7.162	Trans-2-methylpen-ta-1.3-diene	Alkane	C_6H_{10}	1124	-	0.17
9	9.368	Tetradecane	Alkane	$C_{14}H_{30}$	1382	-	0.21
10	9.700	β-Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	1317	1444	0.52
11	9.770	α-Ionone	Norsesquiterpenoid	$C_{13}H_{20}O$	1430	1429	0.25
12	9.999	Ethyl chrysanthemumate	Ester	$C_{12}H_{20}O_2$	1310	-	0.28
13	10.394	Zonarene	Sesquiterpenoid	$C_{15}H_{24}$	1422	1526	0.52
14	10.533	Alloaromadendrene	Sesquiterpenoid	$C_{15}H_{24}$	1500	1457	0.24
15	10.604	α-Zingiberene	Sesquiterpene	$C_{15}H_{24}$	1500	1487	5.12
16	10.673	γ-Muurolene	Sesquiterpene	$C_{15}H_{24}$	1500	1476	1.94
17	10.741	α-Farnesene	Sesquiterpene	$C_{15}H_{24}$	1500	1507	5.70
18	10.765	β-Bisabolene	Sesquiterpene	$C_{15}H_{24}$	1500	1509	3.11
19	10.918	Selina-3,7(11)-diene	Sesquiterpene	$C_{15}H_{24}$	1500	1532	0.36

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20	10.955	β-Sesquiphellandrene	Sesquiterpene	$C_{15}H_{24}$	1520	-	5.27
21	11.105	Hexadecyne	Alkyne	$C_{16}H_{30}$	1386	-	2.06
22	11.349	Lauric acid	Alkanoic acid	$C_{12}H_{24}O_2$	1559	1567	0.29
23	11.700	Caryophyllene oxide	Sesquiterpenoid oxide	$C_{15}H_{24}O$	1519	1583	0.55
24	11.789	Hexadecane	Alkane	$C_{16}H_{34}$	1500	-	0.82
25	12.00	α-Longipinene	Sesquiterpene	$C_{15}H_{24}$	1553	-	0.25
26	12.237	β-Gurjunene	Sesquiterpene	$C_{15}H_{24}$	1500	1442	0.30
27	12.497	Azulene	Sesquiterpene	$C_{15}H_{24}$	1500	1488	0.46
28	12.589	Turmerone	Sesquiterpene	$C_{15}H_{20}O$	1598	-	5.78
29	12.684	Trans-8-Heptadecene	Alkene	$C_{17}H_{34}$	1559	1676	0.79
30	12.912	n-Heptadecane	Alkane	$C_{17}H_{36}$	9831	-	0.58
31	12.996	Curlone	Sesquiterpenoid	$C_{15}H_{22}O$	1973	1701	1.26
32	13.035	n-Heneicosane	Alkane	$C_{21}H_{44}$	1319	-	0.38
33	13.082	1-Methyl-1-cyclodecene	Alkene	$C_{11}H_{20}$	2085	-	0.30
34	13.980	n-Octadecane	Alkane	$C_{18}H_{38}$	1429	-	0.73
35	15.264	n-Nonadecane	Alkane	$C_{19}H_{40}$	1665	-	0.24
36	15.621	Palmitic acid	Alkanoic acid	$C_{16}H_{32}O_2$	1950	1964	3.47
37	15.972	n-Eicosane	Alkane	$C_{20}H_{42}$	1681	-	0.47
38	16.929	Methyl oleate	Ester	$C_{19}H_{36}O_2$	2000	-	2.55
39	17.051	Phytol	Diterpene	$C_{20}H_{40}O$	1855	-	8.92
40	17.202	Linoelaidic acid	Alkanoic acid	$C_{18}H_{32}O_2$	2040	-	0.26
41	17.798	n-Docosane	Alkane	$C_{22}H_{46}$	2090	-	0.25
42	18.025	1-Docosene	Alkene	$C_{22}H_{44}$	2226	2190	0.18
43	18.655	Tricosane	Alkane	$C_{23}H_{48}$	2287	-	0.19
44	19.480	n-Tetracosane	Alkane	$C_{24}H_{50}$	2402	-	0.26
45	20.272	n-Pentacosane	Alkane	$C_{25}H_{52}$	2503	-	0.32
46	21.776	n-Heptacosane	Alkane	$C_{27}H_{56}$	2706	-	0.63
47	23.191	n-Nonacosane	Alkane	$C_{29}H_{60}$	2654	-	7.35
48	24.864	n-Hexacosane	Alkane	$C_{26}H_{54}$	3109	-	0.88
		Total % Composition					75.16

RT = Retention time; RI^a = Kovats retention index (calculated); RI^b = Kovats retention index (Literature).

Table 3: Antioxidant Activity of leaf essential oil of *J. tanjorensis*.

Concentration (µg/mL)	JTE	BHA
1000	93.21 ± 0.02	99.72 ± 0.01
500	93.11 ± 0.00	99.65 ± 0.02
250	92.98 ± 0.02	99.55 ± 0.00
125	92.90 ± 0.01	99.50 ± 0.02

JTE: Leaf essential oil of Jatropha tanjorensis, BHA: Butylated hydroxyanisole, ± = Standard deviation.

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Table 4: Antibacterial activity leaf essential oil of *J. tanjorensis*.

Test Organisms	Gentamicin	Inhibitory Zone (mm) at different Concentration (μg/mL)				
		1000	500	250	125	62.5
Escherichia coli	36 ± 2.00	16 ± 2.00	14 ± 2.00	12 ± 2.00	10 ± 2.00	10 ± 0.00
Pseudomonas aeruginosa	38 ± 0.00	18 ± 2.00	16 ± 0.00	14 ± 0.00	10 ± 2.00	-
Klebsiella pneumonia	38 ± 2.00	16 ± 2.00	14 ± 2.00	12 ± 2.00	10 ± 0.00	-
Staphylococcus aureus	38 ± 2.00	18 ± 2.00	16 ± 0.00	14 ± 0.00	12 ± 0.00	10 ± 0.00
Bacillus subtilis	38 ± 2.00	18 ± 2.00	16 ± 0.00	14 ± 0.00	12 ± 0.00	10 ± 0.00
Salmonella typhi	36 ± 0.00	18 ± 2.00	16 ± 2.00	14 ± 0.00	12 ± 0.00	10 ± 0.00

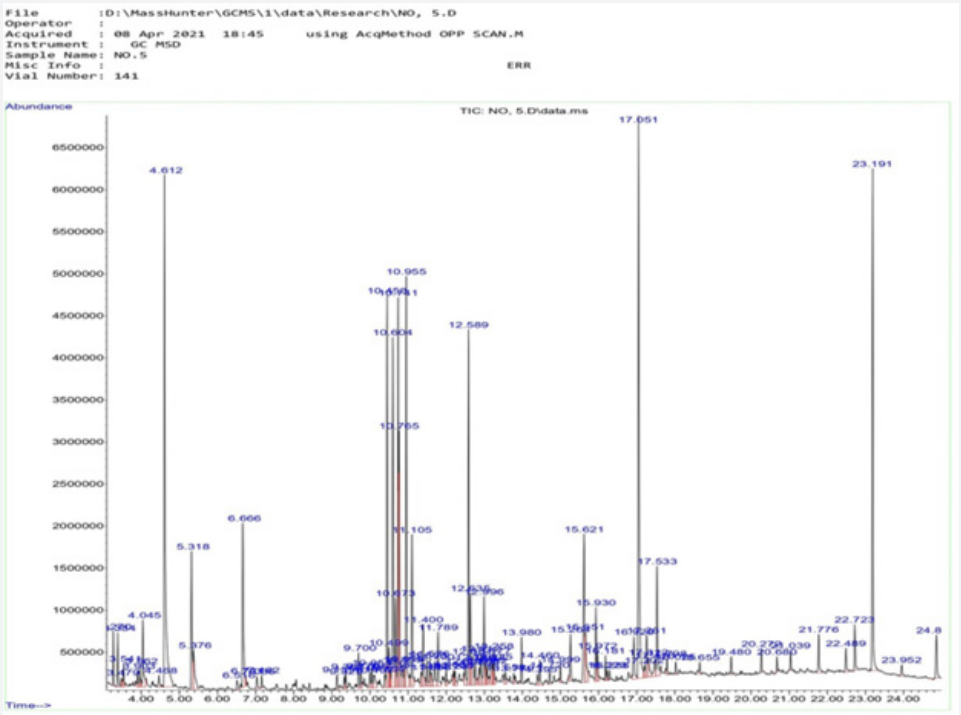

Figure 1: The GC-MS chromatogram of the leaf essential oil of J. tanjorensis.

Discussion

A total of 48 compounds were characterized in the leaf essential oil of *J. tanjorensis* which comprises mainly of terpene compounds (47.84%), n-alkanes (13.73%), and other non-terpene compounds (13.59%). The most abundant compound identified in the oil was phytol (8.92%) and other dominant compounds in the leaf essential oil are n-Nonacosane (7.35%), β-Cis-Ocimene (7.29%), Turmerone (5.78%), α-Farnesene (5.70%), β-Sesquiphellandrene (5.27%) and α-Zingiberene (5.12%). The identified chemical constituents from essential oil *J. tanjorensis* are similar to previously reported compositions of essential oils from plants in the Jatropha genus. A previous study on Jatroph intigrimma, Jatroph gossypifolia and Jatroph roseae Gamal El-Din [15] showed Heneicosane, phytol and nonacosame as the major constituents which were also identified in our study. Previous study on essential oils of Jatropha curcas Adeosun [16], *Jatropha mutabilis* Costa [17] and *Jatropha gossypifolia* Aboaba [18], reported phytol as the major compound; the dominance of sesquiterpenes and oxygenated sesquiterpenes in their essential oils, which is almost relative to our study.

The calculated percentage inhibition shows that the antioxidant activity of leaves essential oils of *J. tanjorensis* and standard (Butylated hydroxyanisole) is concentration dependant.

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The essential oil at all tested concentrations (1000-6.25 µg/mL) exhibited good scavenging ability on DPPH radical which was comparable to Butylated hydroxyanisole. The leaf essential oil exhibited good antibacterial activity at 1000-125 µg/mL with the zone of inhibitory (ZOI) 18 ± 2.00 - 10±2.00 mm, against all the tested organisms as compared to 38±2.00 - 36±0.00 ZOI recorded for Gentamycin; a known antibacterial drug at the same concentrations. The leaf essential oils of *J. tanjorensis* showed sensitivity for Escherichia coli, staphylococcus aureus, Bacillus subtilis and salmonella typhi at all tested concentrations. The leaves and stem essential oils of Jatropha gossypifolia have been reported to have shown potent antibacterial and antioxidant activities Okoh [19].

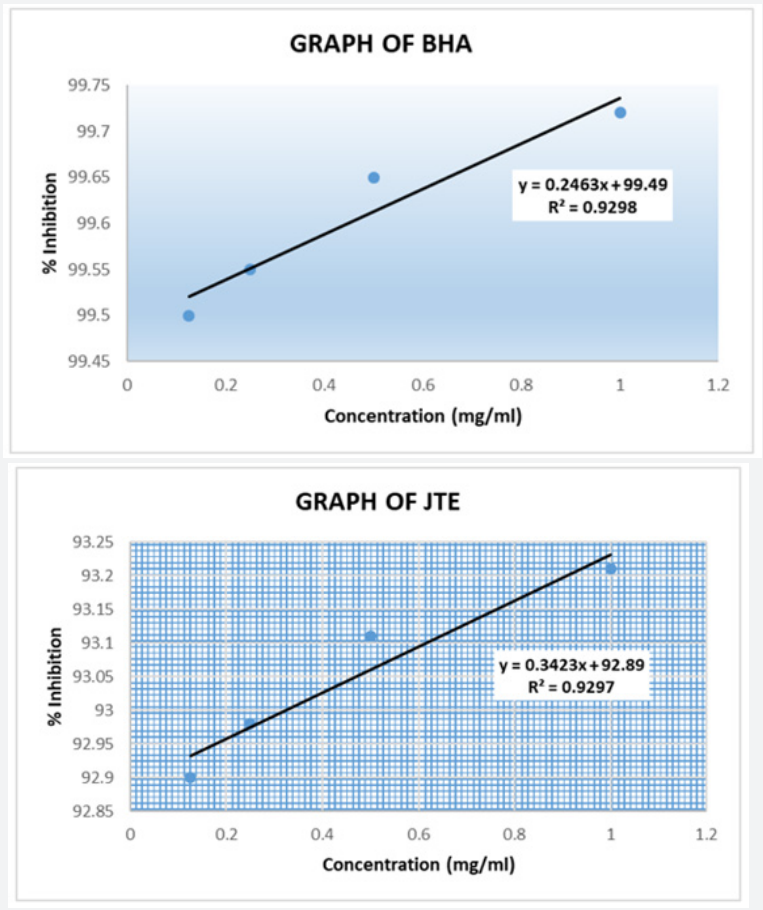

Figure 2: Graphs of % Inhibition of J. tanjorensis leaf essential oil and Standard.

Conclusions

Our study on the chemical composition, antioxidant and antibacterial properties of the essential oil from *J. tanjorensis* leaf is presented here to the best of our knowledge for the first time. The biological properties of *J. tanjorensis* leaf EO serve as the foundation for its pharmaceutical applications.

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