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Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Dalberegia melanoxylon* Tree

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Abstract: Objectives of this work are to investigate and evaluate antimicrobial, phytochemicals, cytotoxicity and antioxidant activities of leaves and bark of different extracts of *Dalberegia melanoxylon*. Methods phytochemical screening and physiochemical properties of the various extracts of *Dalberegia melanoxylon* leaves and bark were carried out using standard methods. Separation of extracts from the bark, using TLC technique with solvents methanol: ethyl acetate: water was carried out. The antimicrobial activity of extracts was assessed on standard organisms; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. Scavenging effect of DPPH radical values of different extracts was used to evaluate the antioxidant activities of these extracts. The obtained results showed that *Dalberegia melanoxylon* extracts from leaves and bark showed the presence of flavonoids, tannins, alkaloids, reducing sugar and glycosides. The plants extracts showed various anti-microbial effects and the highest anti-microbial activity attained by the ethanol extract of the bark against *Bacillus subtilis*. Cytotoxicity studies revealed that the ethyl acetate extract of *Dalbergia melanoxylon* leaves showed a high mortality activity against *Culex quinquefasciatus* larvae while the ethanol extract of the leaves had the highest antioxidant activity. Conclusion the extracts of *D. melanoxylon* exhibits potential antimicrobial and antioxidant properties with moderate cytotoxic effects.

Key words: *Dalberegia melanoxylon* Bark · Leaves · Phytochemicals · Antioxidants · Microorganism

INTRODUCTION

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals and minerals are the basis for treating human diseases [1]. Undoubtedly, the demand for plant-derived products has increased across the world. In the Middle East, Latin America, Africa and Asia more than 85 percent of the populations predominantly rely on traditional medicine, especially on herbal medicines, for their health care needs [2]. One of trees significantly needed for traditional medicine in Africa is *Dalberegia melanoxylon*.

Dalbergia melanoxylon is a small spiny deciduous tree or shrub. Most individuals are 5-7 meters high, multi-stemmed, branched with a low, irregularly shaped crown (Figure1).

Trees can occasionally grow to 20 meters [3-5]. The species occurs in a wide range of woodland habitats on soils that vary from loamy sands to black cotton soils. *Dalberegia melanoxylon* African blackwood occurs in: Angola; Botswana; Burkina Faso; Central African Republic; Chad; Côte d'Ivoire; Democratic Republic of Congo; Ethiopia; Sudan, South Sudan, Kenya; Malawi; Mozambique; Nigeria; Senegal; South Africa; Tanzania; Uganda; and Zimbabwe. The species is also reported to occur in Benin, Cameroon, Eritrea, Ghana, Guinea, Mali, Togo and Zambia. Early reports indicate that the species has been naturalised in India and possibly elsewhere in Asia, although the current status of such populations is unknown [6-8] *D. melanoxylon* is widespread in Sudan, present in Blue Nile, Darfur, -Kassala Algadaraf and South Kordofan. The results obtained from literature showed that

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(a) 

a= General morphology

(b) 

b= Leaves

(c) 

c=bark

Fig. 1: General morphology, leaves and bark of *Dalberegia melanoxylon,* African black wood

Dalberegia melanoxylon bark was exhibited strongest antimicrobial activity and ethanol fraction showed significant antibacterial [9, 10].

The present study aimed to investigate and evaluate the phytochemicals, cytotoxicity antimicrobial and antioxidant activities of the leaves and bark of *D. melanoxylon* tree.

MATERIALS AND METHODS

Plant Material:

Dalberegia melanoxylon leaves and bark were collected from Algoz area- South Kordofan State-Sudan (September 2017) [2].

Identification of the plant Dalberegia melanoxylon plant was identified and authenticated by taxonomists of Medicinal and Aromatic Plants Research Institute, National Center for Research, Sudan. A herbarium sample was deposited in the herbarium of the institute.

Plant Extraction: The leaves and bark of *Dalberegia melanoxylon* were removed from the plant and then washed with running tap water to remove dust and residues. The samples were then air dried for ten days and the leaves were crushed into coarse powder and stored in polythene bags for further use.

Two hundred grams of each dried leaves and bark were weighed, extracted separately in conical flask, placed in magnetic stirrer for four hours at room temperature, then filtered and air dried, the air dried filtrate was subjected to extraction with ethanol, ethyl acetate and n-hexane (1:1). Each extract was filtered through Whatman No.1 filter paper and concentrated. The crude extracts were kept at 20°C in sterile universal bottles.

Physiochemical Properties of Extracts: Moisture content, ash content and crude fibers, reduced, total sugars and yield of *Dalberegia melanoxylon* leaves and bark were determined according to official methods of analysis of AOAC International [12] whereas crude protein was carried out with Kjeldahl procedure [13].

Phytochemical Analysis: Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was carried out on the extracts as described by Evans *et al*. [14], Sofowora [15], Harbone [16], Harborne [17], Santaram and Harborne [18] and Gibbs [19], respectively.

Detection of Alkaloids: About 0.5 g of each extract was stirred with 2 ml of 1% aqueous hydrochloric acid on a steam bath and filtered. 1ml of the filtrate was treated with a few drops of Meyer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Precipitation with either of those reagents was taken as evidence for the presence of alkaloids [14, 15].

Detection of Flavonoids: Two ml of 80% methanol were added to 2 ml of each extract. One ml of KOH was added in one tube, one ml of $ALCl_3$ for second tube, one ml of ammonium hydroxide was added fore third one and few powder of magnesium with HCL to fourth tube with heating, the appearance of yellow and magenta color in test indicated the presence of flavonoids [15].

Detection of Tannins: Two ml of extracts was stirred with 1 milliliter of distilled water, filtered and few drops of ferric chloride were added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins [14, 16].

Detection of Saponins: Two ml of extracts was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins [17, 20].

Detection of Triterpenes and Sterols: Two ml of extracts were dried, dissolved in ten ml of chloroform, few drops of conc. Sulfuric acid were added, if two layers formed, upper green color indicate presence of sterols and lower red brown ring indicate presence of triterpenes [18, 19].

Detection of Cardiac Glycosides: Two ml of the extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution 9 followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycoside [21].

Antimicrobial Activity: Antimicrobial activity of different extracts of the leaves and bark of *Dalberegia melanoxylon* leaves and bark was evaluated against standard organisms; *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida Albicans*. The cup-plate agar diffusion method was adopted with minor modifications to assess the antibacterial activity of the prepared extracts [22-24].

Thin Layer Chromatography TLC: Thin layer chromatography was applied according to the method described by Wagner [25].

TLC procedure was applied to separate the three extracts of *Dalberigia melanoxylon* bark in order to identify the different constituents of the extract depend on RF values and color of spots of reagents. TLC plates were detected with visible light and ultra violet light according to Egon Stahl and Ashworth [26]. The reagents used were Sulfuric acid and vanillin sulfuric acid.

Cytotoxicity Study: *Culex quinquefasciatus egg* rafts were collected from Alkadro area-North of Khartoum State, Sudan. The egg rafts were kept in aluminum dishes 10.6X 10.6 X 1.6 inches with distilled water till hatching. Hatched larvae were fed with fine powdered bread. Dead larvae were continuously removed to avoid contamination.

RESULTS

The results of general physiochemical analysis of *Dalberegia melanoxylon* African black wood of leaves are presented in (Table 1). Physiochemical properties of the leaves indicated the presence of fibers (17.4%), moisture content (13%) and ash content (7.4%). The reducing sugars amount was (4.5%) and total sugars were (15.2%).

Physical properties of *Dalberegia melanoxylon*, bark extracts revealed that n-hexane extract had green color, semi oily consistency and trace yield (0.6%), while extract of ethyl acetate was solid with dark green color. On the other hand, it was found that the methanol extract yielded (5.2%), solid and dark in color (Table 2).

Table 1: Physiochemical component of *Dalberegia melanoxylon* leaves.

Component (%)	Measured value (%)	± standard deviation
Moisture content	13	0.1
Ash	7.4	0.1
Crude protein	6.3	0.2
Crude fibers	17.4	0.3
Reducing sugars	4.5	0.2
Total sugars	15.2	0.4

Table 2: Physical characteristics of *Dalberegia melanoxylon* bark extracts

Type of solvent	Yield (%)	Color of extracts	Consistency of extracts
n-hexane	0.6	green	Semi-oily
Ethyl acetate	2.6	Dark green	Solid
Ethanol	5.2	Black	Solid

Table 3: Phytochemical screening of *Dalberegia melanoxylon* leaves

		Successive extraction with		
Secondary metabolites	Tests	n-hexane	Ethyl acetate	ethanol
Alkaloids	Mayer's	-	-	-
	Drogndroff's	-	-	-
	Hager's	-	-	-
	Wagner's	-	-	-
Flavonoids	NaOH	-	-	-
	NH_4OH	-	-	-
	$ALCL_3$	-	-	-
	Mg/H_2SO_4	-	-	-
Saponins	Foam	-	+	+++
Tannins	$FeCl_3$	-	+	+++
	Gelatin	-	+	+++
Sterols/Triterpene	Liebermman's	++	++++	+++
	Salkowski	++	++++	+++
Glycosides	Cardic	+	+	+

++++ = very high concentration
+++ = high concentration
++ = moderate concentration
+ = trace amount
-= absent

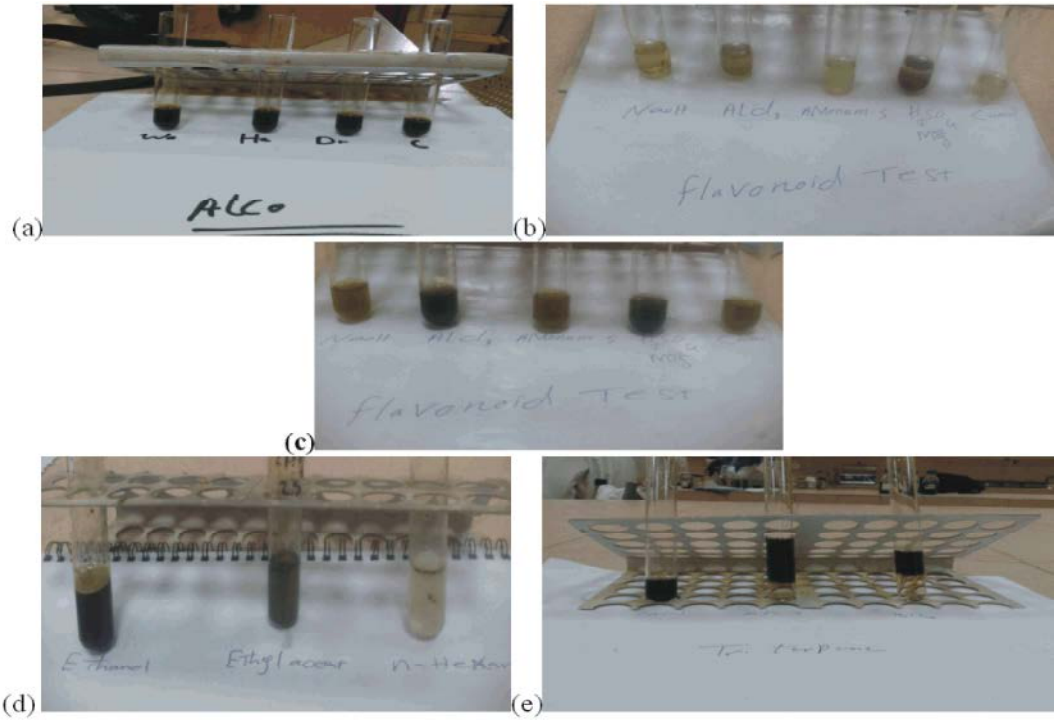


Fig. 2: Phytochemical screening of *Dalberegia melanoxylon,* African black wood, leaves
a) alkaloid for the different extracts; b) flavonoids extracts of n-Hexane of leaves;
c) flavonoids extracts of ethyl acetate; d) Saponins the different extracts; e. Sterol/triterpenes.

Phytochemical studies carried out on the extracts of *Dalberegia melanoxylon* leaves were totally absent of alkaloids and flavonoids with presence of tannins, saponnins, sterols/triterpene and glycosides in various concentrations (Table 3 and Figure 2). Flavonoids, alkaloids sterols/triterpene, glycosides and tannins were present in the bark of *Dalberegia melanoxylon* with absence of saponins (Table 4 and Figures 3-4).

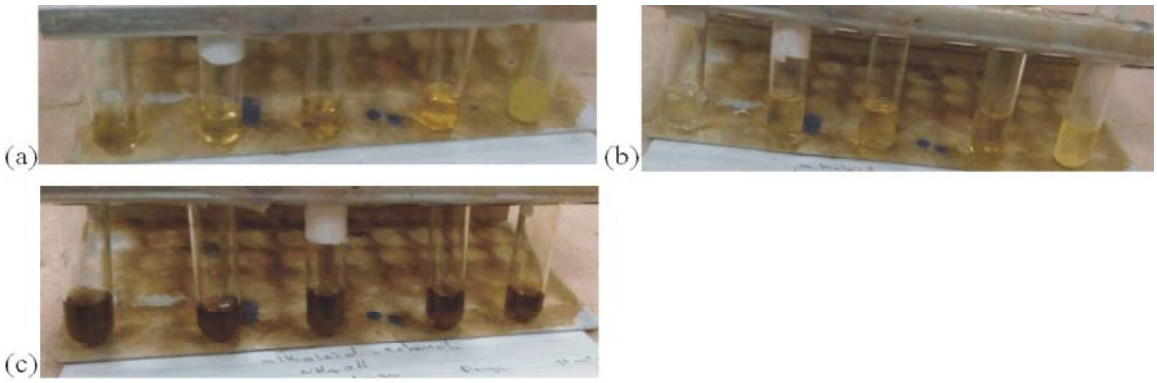
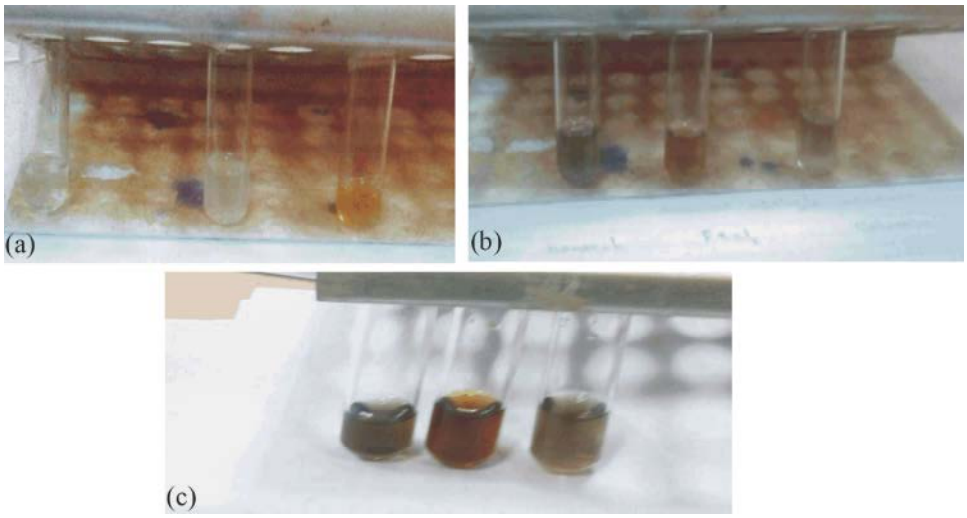


Fig. 3: Phytochemical screening of alkaloid for the bark of *Dalberegia melanoxylon,* African black wood. a) n-hexane extract; b) ethyl acetate extrac



Control $FeCl_3$ test Gelatin

Fig. 4: Phytochemical screening of tannins for the bark of *Dalberegia melanoxylon,* African black wood. a) n-hexane extract, b) ethyl acetate extract, c) ethanol extract.

Table 4: Phytochemical screening of *Dalberegia melanoxylon* bark

		Successive extraction with		
Secondary metabolites	Tests	n-hexane	Ethyl acetate	ethanol
Alkaloids	Mayer's	++	++	-
	Drogndroff's	+++	+++	-
	Hager's	+++	+++	-
	Wagner's	+	+	-
Flavonoids	NaOH	+++	++	+++
	NH_4OH	++	++	+++
	$ALCL_3$	+	++	+++
	Mg/H_2SO_4	+++	-	+++
Saponins	Foam	-	-	-
Tannins	$FeCl_3$	+++	+++	+++
	Gelatin	+++	++	++
Sterols/Triterpene	Liebermman's	+++	+++	+++
	Salkowski	+++	+++	+++
Glycosides	Cardic	+++	++	++

+++ = high concentration
++ = moderate concentration
+ = trace amount
= absent

Table 5: Scavenging effect of DPPH radical values of different extracts of *Dalberegiamelanoxylon* leaves

Extracts	DPPH activity,%	IC 50
n-hexane	11.83	-
Ethyl acetate	20.35	-
Ethanol	76.52	13.793 µg mL^{-1}

IC 50 = the concentration of the samples required to scavenge 50% of the peroxide radical

Table 6: African black wood: Separation of extracts from *Dalberegia melanoxylon* bark, using TLC technique with solvents methanol: ethyl acetate: water (1:1).

	After spray with vanillin under UV 250 nm	
Extracts	RF	Color
Ethanol	0.21	red
	0.55	white
	0.74	
	0.85	blue
Ethyl acetate	0.54	
	0.71	white
	0.67	
	0.86	white
n-hexane	0.02	blue
	0.09	
	0.2	
	0.5	white

Table 7: Antimicrobial activity of the crude extracts of *Dalberegia melanoxylon* bark at different concentrations against tested organisms

		Tested organisms applied in diameter zone of inhibition, mm				
Extracts	Concentration mg mL^{-1}	*Escherichia coli* gram -ve Gram-ve bacteria	*Pseudomonas aeruginosa* gram –ve bacteria	*Staphylococcus aureus* gram +ve bacteria	*Bacillus subtilis* gram +ve bacteria	*Candida albicans* fungal
n-hexane	100	13	14	15	18	-
	50	12	13	14	17	-
	25	11	12	12	15	-
Ethyl acetate	100	16	13	16	21	17
	50	15	12	15	20	16
	25	14	11	11	19	15
Ethanol	100	19	19	18	22	17
	50	18	18	17	20	14
	25	17	16	16	19	12

Sensitive (Active) >18 mm
Intermediate (Partial active) 14-18 mm
Resistance (Inactive) < 14

TLC revealed that the n-hexane extract had RF values as follows: (0.02, 0.09, 0.2, 0.5), ethyl acetate extract had RF values: (0.54, 0.67, 0.71, 0.86) and ethanol extracts RF values: (0.21, 0.55, 0.74, 0.85).

Cytotoxicity studies revealed that the ethyl acetate extract of *Dalbergia melanoxylon* leaves showed high mortality activity against *Culex quinquefasciatus* larvae at a dose concentration of 1000 ppm followed by 100 ppm. The IC 50 was detected at 308.04 µg mL^{-1}.

Anti-oxidant Activity: n-hexane and ethyl acetate leaves showed a low anti-oxidant activity with percentages of 11.83% and 20.35% respectively while the ethanol extract had showed a high antioxidant activity (76.62%) with IC50 value 13.79.79 µg mL^{-1} (Tables 5 and 6).

The results of antimicrobial activity of different extracts of *Dalberigia melanoxylon* are presented in (Table 7). The highest anti-microbial activity was attained by Ethanol extract of the bark against *Bacillus subtilis* and a moderate activity against *Staphylococcus aureus* and *Candida Albicans* While the ethyl acetate extract had moderate activity *Candida albicans* and moderate activity against both *Escherichia coli* and *Staphylococcus aureus*. However, it was not active against both *Bacillus subtilis* and *Pseudomonas aeruginosa*.

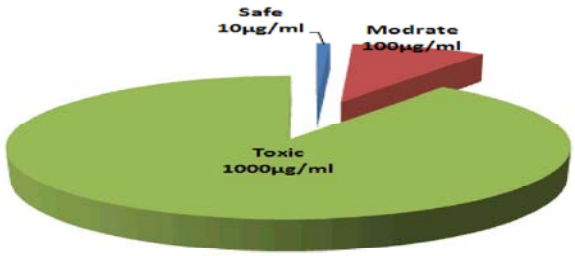


Fig. 5: The mortality of *Culex quinquefasciatus* larvae with ethyl acetate extract of *Dalbergia melanoxylon* African black wood, leaves

DISCUSSION

The physiochemical analysis of *Dalbergia melanoxylon* leaves revealed that this plant is as an excellent source of fiber and moisture content with ash value rich of minerals. The total sugars amount was high.

In the present study, the extracts of *Dalbergia melanoxylon* contained high amounts of flavonoids, tannins, sterols/ triterpenes and glycosides. It seemed that concentrations of the phytochemicals found in the bark of *D. melanoxylon* were higher than those found in the leaves. The occurrence of isoflavones, neoflavones, sterols, anthraquinones, cinnamyl esters and triterpenes in the genus Dalbergia in which *D. melanoxylon* belongs has previously been reported. The active principles responsible for the therapeutic effects of medicinal plants are phytochemicals, typically secondary metabolites, including but not limited to alkaloids, steroids, flavonoids and tannins [27]. Thus, chemical constituents found in this plant, ascertain its potential medicinal value.

LD_{50} (The amount of a toxic agent (As a poison, virus, or radiation) that is sufficient to kill 50 percent of a population of animals usually within a certain time – called also median lethal dose). In the present study, the leaves of *D. melanoxylon* extracted with ethyl acetate were tested against *Culex quinquefasciatus* larvae and showed a moderate activity with LD_{50} estimated by 100 µg mL^{-1} (Figure 5).

The ethanol extracts of *D. melanoxylon* bark at concentration 100 mg mL^{-1} was revealed higher inhabitation zones against four bacterial *P. aeruginosa* (19), *E. coli* (19), *B. subtilis* (19), *S. aureus* (18) and fungus *C. albicans* (17) than reference drug Amoxicillin, but less than 40 µg mL^{-1} of Ketoconazole and Iitraconazole. This higher activity due to presence of flavonoid and other phytochemicals. The ethyl acetate at same concentration of the bark showed the same activity of inhabitation zone against bacteria *B. subtilis* (21) and higher activity against fungus *B. subtilis* (16), *E. coli* (16) than 40 µg mL^{-1} of Ampicillin and Amoxicillin. It was also higher activity against all bacteria and fungus *C. albicans* compared to 40 µg mL^{-1}Ketoconazole and Iitraconazole. In n-hexane extract of bark showed less activity against all bacteria and fungus at 40 µg mL^{-1} of Ciprofloxacin, Amoxicillin and Gentamicin.

In accordance to the DPPH method, the ethanolic extract of the leaves has shown the highest anti-oxidant activity which initiates its potency in the treatment of many ailments.

CONCLUSION

The ethanol extracts of leaves and bark of *D. melanoxylon* used in this study showed the presence of variable and significant phytochemicals and the bark of this plant has exhibited a potential antimicrobial activity against tested standard microorganisms. The leaves extracts has shown a moderate anti-oxidant and cytotoxic activities. In general the bark extracts has better chemical characteristics than the leaves. Further studies to evaluate other beneficial properties exhibited by this valuable tree are highly recommended.

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Abbreviations:

DPPH organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl

RF value in chromatography, the retardation factor is the fraction of an analyte in the mobile phase of a chromatographic system

LD_{50} Lethal Dose, 50%" or median lethal dose. It is the amount of the substance required (usually per body weight) to kill 50% of the test population.

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