RESEARCH ARTICLE

Identify Bioactive Compounds by Gc-Ms From the Highest Antimicrobial Extract from Roots and Leaves of *Elephantopus Scaber*

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ABSTRACT

Medicinal plants are used traditionally in the treatment of various kinds of diseases since time immemorial. The present study was carried out to evaluate the antimicrobial activities of roots and leaves of *Elephantopus scaber*. The ethyl alcohol extracts of roots and leaves were subjected to phytochemical analyses, which revealed the presence of alkaloids, phenols, flavonoids, saponins, steroids, tannins, coumarins, quinones and glycosides in most of the selected of roots and leaves. Roots and leaves were investigated for in vitro antimicrobial activity against *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida albican* by well diffusion method by using a different concentrations (20, 40, 60, 80, 100)mg/mL. Roots extract showed promising antimicrobial activity against all test organisms. The root has the best activity against all test organisms compared with leaves extract. The minimum inhibitory concentration (MIC) of the roots and leaf extracts were also determined against different test organisms. The MIC value of roots and leaves extract ranged from 100 mg/mL to 500 mg/mL. This study showed the extract which gave higher efficacy is roots extract where it was detected the bioactive compounds in this extract by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of the active samples confirmed the presence of compounds containing -OOH, -OH, -N, and –S groups, which are associated with bacterial inhibition in conventional antibiotics. The 10 major constituents obtained from samples suspected to contain antibacterial activity include Methanehydrazonic acid, N-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester (8.87%); 2-Butenoic acid, 3-methyl-, methylester (0.60%); Ethanone,1-cyclopropyl-2-[3-pyridinyl]- (0.73%); 6-Octen-1-yn-3-ol, 3,7-dimethyl- (1.11%); 1,5-Dimethyl-2-pyrrolecarbonitrile (0.61%).

Keywords: Antimicrobial Extract, Bioactive Compounds, Elephantopus Scaber, Roots

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INTRODUCTION

The conti.nuous spread of multi-drug-resistant patho gens has become a threat to public health and a major concern for infection control practitioners worldwide.[1] In addition to increasing the cost of drug regimens, this scenario has paved way for re-emergence of previously controlled diseases and has contributed substantially to high frequency of opportunistic and chronic infection cases in developing countries.[2] Some of the pathogens include bacteria, viruses, fungi, and prion.[1] Bacteria cause a wide range of infections, resulting in mild to life-threatening illnesses that require immediate interventions.[3] Common ba cterial infections include respiratory infections, ear infections, gastrointestinal infections, and skin disorders.[4-5] In developing countries, outbr eaks of bacterial infections occur frequantly in congested a reas such as refu gee camps, slums and in a reas with high population density. Food ven dors, slum d wellers, riparian communities, fishermen, and school children are among the r isk groups.[6,7] Antibiotics such as ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole (TMP-SMX), amoxic illin, and ciprof loxacin have been co mmonly used to treat bac terial infections.[8] The bioactive parts against bacteria in these conventional a ntibiotics include structural moieties that include -Cl, -F, -N, -NH2, -S, -COOH and –OH, which are also found in many herbs used traditionally against b acterial infections. Studies have sho wn that sulfur-containing compou nds have strong inhibitory antibacterial activities.[9-11] Nitrite has toxic properties while nitrous acid is ba ctericidal, chlorine releasing compounds such as chlorine dioxide (ClO_2), acidic and alcoholic compounds act as antibacterial agents.[12] Some vegetable tri als have been reported to be as effective as conventional treatmen ts and provide therap y for bacterial infections.[3] Herbs, foods and spices contain many com pounds, and it is often un clear which o ne is associa ted with beneficial effec ts. The compounds in drugs vary in different spec ies. However, even within a single species the phytochemical

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composition may be affected by the plant's growing conditions and different p arts of a herb can have di fferent chemical compositions.[13] Elephantopus scaber L. (Elephant's foot) is an important medicinal plant that is distributed worldwide in all tropical regions.[14] The whole plant (root, leaf, and bark) is used medicinally, because of the pres ence of many bioactive compounds throughout the entire plant.[15,16] As per the traditional system of medicine, it is reported that the roo ts are used as for their antipyretic, cardiotonic, and diur etic activities.[17] A decoction of the roots and leaves is used as an emo llient and given in dysuria, diarrhea, dysentery, and for stomach pain.[18] The aqueous extract of leaves is ap plied externally to treat eczema and ulcers.[19] The whole plant is macerated and applied on the surface of wounds to promote wound healing a ctivity.[20]

MATERIALS AND METHODS

Collection the plant

The selected *Elephantopus scaber L.* was washed thoroughly and separated roots and leaves from plants then dried under shade. The dried plant material was grounded into a fine powder). The powdered material was extracted in Soxhlet apparatus for 24 hours ethyl alcohol solvents. The solvent was then evaporated using Rotary evaporator at 4°C for further use.[21]

Prepare the Antimicrobial Samples

Before antimicrobial assay stock concentration of 200mg/mL extract was prepared in 0.25% DMSO (Dimethyl sulfoxide) and was filtered through 0.45-micron cellulose acetate membrane filter (Sartorious). With the filtrate, further dilutions were made to get the concentrations of (100, 80, 60, 40 and 20) mg/mL, which were then used for the antimicrobial assay.

Screening of antimicrobial activity of root and leaves against test organisms (well diffusion method)

The antimicrobial assay was performed by agar well diffusion method: Method established by the National Committee for Clinical Laboratory Standard.[21]

About 20 mL of the nutrient agar medium was poured into the Petri plates and was left to solidify. To the solidified medium, 100 µL of bacterial suspension was added and was spread uniformly with the glass spreader. Four wells were prepared in the plates with the help of a cup-borer (0.8 cm). Into the tow wells, 100 µL of the plant extract (test compound) was introduced and in one well 100 µL of 0.25% DMSO (negative control) was introduced. The plates were then incubated overnight at 37°C. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, a negative control is maintained where pure solvents are used instead of the extract.[22] The experiment was performed four times, and the mean values were presented.

For the evaluation of the synergistic antimicrobial activity of root and leaf in the combination of tow, a suitable diffusion method was followed. Four wells were prepared in the plates. Into tow opposite well, each extract (50 µL +50 µL) was added, and in another opposite well individual extract was added. After incubation at 37°C for 24 hours, the observation was made. The zone of inhibitions formed by the combination of the extract was compared with the zone formed by the individual extract.

2.4 Determination of MIC (Minimum Inhibitory Concentration) of plant extract by Microdilution Method

The lowest concentration or highest dilution of the plant extract that inhibits the visible growth of test microorganisms is known as minimum inhibitory concentration.[23] The standardization of the bacterial cell number or preparation of the bacterial suspension is of critical importance for susceptibility testing and obtaining accurate minimum inhibitory concentration. The minimum inhibitory concentration assay was performed by using agar dilution method.[23]

Agar dilution method

Different concentrations of plant extract were prepared. Around 20mL of nutrient agar was prepared in each test tube and after autoclaving at 121°C, 15 lb for 15 minutes, medium was allowed to c ool at 45-50°C, then different concentrations of plant extract were added in the respective test tubes to make the final conc entration of 1-5 mg/mL, which was then poured into the Petri pla tes after mixing properly. The plates were left to solidify. Stan dard inoculums of test orga nisms were prepared. Test microo rganisms were s potted in the agar plates with the help of micropipette set at 1µL to deliver the spots.[23] Control experiments were also done to see the effect of antibi otic and sol vent alone (without plant extract) on the gro wth of all the test or ganisms.[24] The Plates were incubated at 37°C for 24hrs. The lowest concent ration of the plant extracts that inhibits the growth of test microo rganism was considered as the MIC of the extract.[23]

GC-MS analyses

Samples of 1.0 g extracts were dissolved separately in 2 mL of DCM. It was shaken and mixed using the ultrasound path for 3 minutes, then filtered using glass wool. The sample was drawn into small vials, and then 1 µL was injected into the GC-MS. The resultant mixture was left overnight, filtered using glass wool and 5 µL of the filtrate was dissolved in 1 ml of pentane. The sample (1 µL) was injected into 52 the GC-MS for analysis then 1 µL was analyzed.

RESULT AND DISCUSSION

Phytochemical analysis of roots and leaves

The analysis of phytochemicals of roots and leaves extract revealed the presence of various phytoconstituents. The results are presented in Table 1. Whereas all the tested phytochemicals were found in ethyl alcohol extract of root expect Quinones.The analysis of phytochemical of leaves for ethyl alcohol revealed the presence of (Alkaloids, Phenols, Saponins, Steroids, glycosides and Coumarins.

Table 1: phytochemical analysis of roots and leaves extracts

Bioactive compounds	*E. scaber root*	*E. scaber leaf*
Alkaloids	+	-
Phenols	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	+	+
Tannins	+	-
Coumarins	+	+
Quinones	-	-
Glycosides	+	+

Antimicrobial activity

The result representing the antibacterial activity of root extract from *E. scaber* against microorganisms is presented in Table 2. The highest activity of plant extract has been shown in root extract and was found to be 22 mm diameter of zone of inhibition against *S.aureus* at the concentration of 100 mg/ml followed by 19 mm diameter of zone of inhibition against *S. tphis* and *S. pyogenes* at concentration of 100 mg/mL then followed by 18 mm diameter of zone of inhibition against *Escherichia coli* concentration of 100 mg/mL. In comparison to Gentamicin at 100 mg/mL, as shown in Table-2. Root extract of *E. scaber* possesses significant antibacterial activity at a concentration of 100 mg/ml compare with leaves extract.

Leaves extract from *E. scaber* has been assessed against microorganisms is presented in Table 2. The highest activity of plant extract has been shown in leaves extract and was found to be 20 mm diameter of zone of inhibition against *S.aureus* at the concentration of 100 mg/ml followed by 18 mm diameter of zone of inhibition against *S. tphis* then 17mm against Escherichia coli at concentration of 100 mg/ml then followed by 16 mm diameter of zone of inhibition against *S. pyogenes* concentration of 100 mg/mL. In comparison to Gentamicin at 100 mg/mL as shown in Table 2.

Finding indicated in Table 3, the *Elephantopus scaber* for roots extracts showed weak effective of *Klebsiella pneumoniae*, where the roots extract showed at the concentration of (100) mg/mL zone of inhibition (6)mm. Table 3 showed for roots extracts the zone of inhibition against *Candida Albican* at concentration of (100)mg/ml the largest zone inhibition (15) mm was observed.

Leaves extract of *Elephantopus scaber* in Table-3 has been a weak effective against *Klebsiella pneumoniae.* Where the leaves extract showed at the concentration of (100)mg/ml zone of inhibition (3)mm, leaves extracts found the largest zone inhibition of (14)mm against *Candida Albican* at a concentration of (100)mg/mL.

All the result compared with Gentamicin 100 mg/ml as standard Figure 1 and 2.

The minimum inhibitory concentration (MIC) assay of roots and leaves extract against six test organisms

Table (3) shows the Minimum inhibitory concentration of roots extract from Elephanthopus scaber. The MIC value of root extract was found to be 100 mg/ml for *Escherichia coli , Salmonella typhimurium Streptococcus pyogenes, Staphylococcus aureus* and *Condida Albican*, and extracts exhibited an MIC of 300mg/ml against *Klebsiella pneumoniae.* The MIC value of leaves extract was found to be 100 mg/ml for *Escherichia coli , Salmonella typhimurium Streptococcus pyogenes, Staphylococcus aureus* and *Condida Albican*, and extracts exhibited an MIC of 300mg/ml against *Klebsiella pneumoniae.* Table 3: Minimum inhibitory concentration of roots and leaves from *E. scaber* and standard antibiotics against gram positive and gram negative and Fungus organism.

Gas chromatography-mass spectrometry analyses

Gas chromatography-mass spectrometry (GC-MS) analyses

The research showed that the highest influence is ethyl

Table 2: Zone of inhibition of *E. scaber* extracts and standard antibiotics against four microorganisms

		Zone of Inhibition (mm)																			
	Extr.	*Escherichia coli*					*S.aureus*					*S. pyogenes*					*S. tphis*				
SN	*code*	*20*	*40*	*60*	*80*	*100*	*20*	*40*	*60*	*80*	*100*	*20*	*40*	*60*	*80*	*100*	*20*	*40*	*60*	*80*	*100*
1	Root	5	10	14	16	18	6	15	18	20	22	6	10	16	17	19	5	9	15	17	19
2	Leaf	4.3	7.4	12	15	17	4	9	16	18	20	5	10	12	14	16	4	9	14	16	18
Positive and negative control																					
4	GE	28.25				27.5					28.5					27.3					
5	DM	0				0					0					0					

GE: Gentamicin 100 mg/ml; DM: DMSO (0.25%);

Table 3: Zone of inhibition of *E. scaber* extracts and standard antibiotics against tow microorganisms

		Zone of Inhibition (mm)									
		K. pneumonia					*Candida Albican*				
SN	*Extr. code*	*20*	*40*	*60*	*80*	*100*	*20*	*40*	*60*	*80*	*100*
1	Root	-	-	2	4.5	6	5	7	9	12	15
2	Leaf	-	-	-	-	3	4.5	7	10	12	14
Positive and negative control											
4	GE	20				GF		22			
5	DM	0				0					

GE: Gentamicin 100 mg/ml; DM: DMSO (0.25%); GF: Griseofulvin (100 µg/ml)

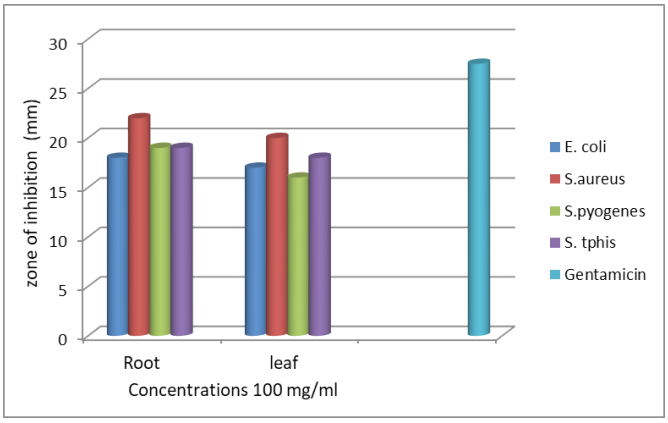

Figure 1: Antimicrobial activity of roots and leaves extracts against microorganisms compared with Gentamicin 100 mg/ml

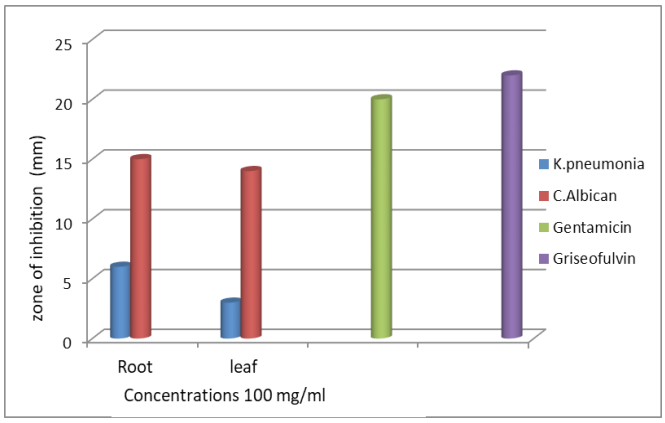

Figure 2: Antimicrobial activity of roots and leaves extracts against microorganisms compared with Gentamicin and Griseofulvin 100 mg/ml

alcohol extract of the roots compared with other extracts from *Elephantopus scaber.* Chemical compounds in the extract was also determined by GC-MS.

Roots extract of Elephantopus scaber

Roots extract from *Elephantopus scaber* was analyzed by GC-MS, and the sample gave a chromatogram having several peaks. The suspected antibacterial compounds with their molecular formula and weight are listed in Table 4. The chromatograms obtained from ethyl alcohol extract of roots with peaks representing suspected antibacterial compounds are shown in Figure 3.

Minimal inhibition concentration (µg/mL)

The microbial	*The extract*		*Standard Antibiotics*	
	Root	*Leaves*	*GE*	*CI*
Escherichia coli	100	100	25	20
Streptococcus pyogenes	100	200	50	25
Salmonella typhimurium	100	100	25	20
Staphylococcus aureus	100	100	50	25
Klebsiella pneumoniae	300	300	100	50
			GR	NY
Fungus Condida Albican	100	100	100	50

Table 4: The GC-MS profile of compounds suspected to contain antibacterial properties identified in roots from *Elephantopus scaber*

					Relative %
No	*Compound*	*Molecular formula*	*M+ (g/mol)*	*Retention time (min)*	*Root extract*
1	Pyrrolo[2,3-b] indole	$C_{14}H_{16}N_2O_4$	218	20.502	0.73
2	Methanehydrazonic acid, *N*-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester	$C_6H_9N_4OS_2$	218	21.331	8.87
3	Selenourea, phenyl-	$C_7H_8N_2Se$	200	21.531	0.17
4	Imidazole, 4-methyl-5-[3,3,3-trifluoropropionylpropyl]-	$C_{10}H_{13}F_3N_2O$	234	22.248	0.47
5	1,6,10-Dodecatriene-3 ol,3,7,11-trimethyl-	$C_{15}H_{26}O$	222	22.358	0.48
6	2-Butenoic acid, 3-methyl-, methylester	$C_6H_{10}O_2$	114	22.43	0.60
7	2-Azabicyclo[3.2.1]octan-3-one	$C_7H_{11}NO$	125	22.58	0.12
8	2,4-Quinolnediol	$C_9H_7NO_2$	161	22.724	0.44
9	3-[4-Hydroxybenzoylhydrazono]-*N*-mesitylbutyramide	$C_{20}H_{23}N_3O_3$	353	22.974	0.29
10	Phthalic acid, cyclohexylmethyl-3-phenylpropylester	$C_{24}H_{28}O$	380	23.14	0.53
11	Linalool	$C_{10}H_{18}O$	154	13.066	0.05
12	Terpinen-4-ol	$C_{10}H_{18}O_4$	154	14.563	0.25
13	Bicyclo[3.2.2]non-8-en-6-ol, (1R,5-cis,6-cis)-	$C_9H_{14}O$	138	16.105	0.03
14	Guaiacol<para-vinyl->	$C_9H_{10}O_2$	150	16.377	0.07
15	*N*-(2-Phenylethenyl)acetamide	$C_{10}H_{11}NO$	161	17.266	0.03
16	Ethanone,1-cyclopropyl-2-[3-pyridinyl]-	$C_{10}H_{11}NO$	161	19.5	0.73
17	1,5-Dimethyl-2-pyrrolecarbonitrile	$C_7H_8N_2$	120	20.104	0.61
18	6-Octen-1-yn-3-ol, 3,7-dimethyl-	$C_{10}H_{16}O$	152	20.207	1.11
19	Ethyl homovanillate	$C_{11}H_{14}O_4$	210	23.353	0.47
20	Ezlopitant , dehydro-	$C_{32}H_{24}N_2O$	452	32.758	0.14
21	Phenol, 4-pentyl-	$C_{11}H_{16}O$	164	33.341	0.76
22	[1,3,5]Triazine-2,4-diamine,6-	$C_9H_{13}N_7$	219	34.608	0.21
23	*O*-methoxy- α,-methylbenzyl alcohol	$C_9H_{12}O_2$	152	36.307	0.22

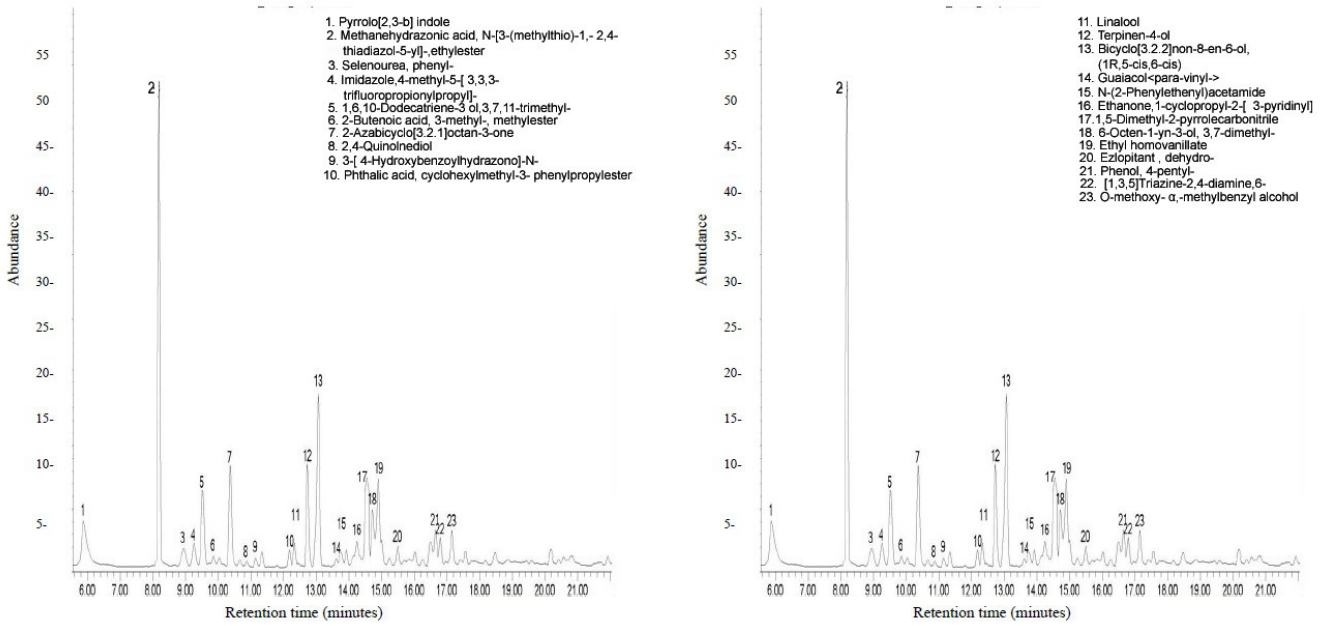

Figure 3: GC-MS Chromatogram obtained of ethyl alcohol extract from Elephantopus scaber L.

Roots extract from Elephantopus scaber had a wide range of suspected antibacterial components including; pyrrolo [2,3-b] indole (0.73%); Methanehydrazonic acid, *N*-[3-(methylthio)-1,-2,4-thiadiazol- 5-yl]-,ethylester (Figure 4.19) (8.87%) ; Selenourea, phenyl- (0.17%);Imidazole, 4-methyl-5- [3,3,3-trifluoropropionylpropyl]- (0.47%); 1,6,10-Dodecatriene-3 ol,3,7,11-trimethyl- (0.48%); 2-Butenoic acid, 3-methyl-, methylester (0.60%);2-Azabicyclo[3.2.1] octan- 3-one (0.12%); 2,4-Quinolnediol(0.44%); 3-[4-Hydroxybenzoylhydrazono]-*N*-mesitylbutyramide (0.29%) ; Phthalic acid, cyclohexylmethyl-3-phenylpropylester (0.53%); Linalool (0.05%); Terpinen-4-ol (0.25%); which may have confered bacterial inhibition property to this terpene (Fisher and Phillips 2006). Roots extract from *Elephantopus scaber* revealed the presence of Bicyclo[3.2.2] non-8-en-6-ol, (1R,5-cis,6-cis)- (0.03%); Guaiacol<para-vinyl->(0.07%); *N*-(2-Phenylethenyl) acetamide (0.03%); Ethanone,1-cyclopropyl-2-[3-pyridinyl]- (0.73%); 1,5-Dimethyl- 2-pyrrolecarbonitrile(0.61%) ; 6-Octen-1-yn-3-ol, 3,7-dimethyl- (Figure 4.19) (1.11%); Ethyl homovanillate (0.47%); Ezlopitant , dehydro- (0.14%) ; Phenol, 4-pentyl- (0.76%); [1,3,5]Triazine-2,4-diamine,6- (0.21%) and *O*-methoxy-α,-methylbenzyl alcohol (0.22%) .

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