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DETERMINATION OF THE PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIAL PROPERTIES OF A RARE AND ENDEMIC PLANT *XANTHOPHYLLUM ARNOTTIANUM* WIGHT OF WESTERN GHATS, INDIA

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ABSTRACT

Xanthophyllum arnottianum Wight is a rare and endemic plant and is having limited distribution in biodiversity hot spot of Western Ghats of India. The study was conducted in the year 2023 to document the phytochemical constitution, antioxidant potential and antimicrobial properties of the leaves of this plant. Study revealed that *Xanthophyllum arnottianum* Wight is a rich source of many of the phytochemicals of which flavonoids composition was found to be 0.08mg/ml-1, tannins 0.57mg/ml-1. Total antioxidant capacity was found to be 0.33mg/ml-1 and enzymatic antioxidants (peroxidase-0.12mg/ml-1). GCMS analysis identified ten principal in the leaf extracts of *X. arnottianum*. This species of plant is recognized for its significant phytochemical profile of importance such as 3,5-Dimethoxyacetophenone, Neophytadiene, Phytol and Acetate. Antimicrobial properties of the methanolic extract of the plant indicated that this plant possesses antibacterial activity against all the three tested microorganisms (*Klebsiella pneumoniae* (8mm), *Staphylococcus aureus* (20mm) and *Escherichia coli* (7mm). This research serves as an initial endeavour that shed light into the wealth of novel biomolecules that are remained as hidden in the plant kingdom.

Keywords: Antioxidants, Antimicrobial propertIes, Endemic plants, Phytochemicals, GCMS

INTRODUCTION

Phytochemicals are the compounds produced by plants as secondary metabolites that may exhibit therapeutic effects and this includes alkaloids, glycosides, terpenoids and phenols. Phytochemicals in plants can be used for their therapeutic purposes or as precursors for the development of new pharmaceuticals. Plants used for the treatment of various diseases contribute to the discovery of new pharmaceuticals and more than 20,000 plant species used in traditional medicines. Many of the active compounds derived from medicinal plants can be effective in the treatment of various infectious diseases that affects humans and animals. Plants possess many free radical scavenging molecules like phenolics, flavonoids and secondary

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metabolites that possess antioxidant properties. Of the important phytochemicals in plants, the alkaloids exhibit important pharmacological uses such as antibacterial, antimalarial, anticancer properties, tannins which are antiseptics in nature, flavonoids exhibit anti-allergic, anti-inflammatory, antioxidant, antimicrobial properties; saponins help plants against microbial attack. Terpenoids are aromatic and used in foods and pharmaceutical industries while the phenols are precursor to a large array of drugs. Antioxidants that possess free radical scavenging capabilities have role in prevention and therapeutic role in many diseases.

As phytochemicals are secondary metabolites integral to the plant's defence mechanisms. Phytochemicals help the plants to protect it from various microorganisms, insects and herbivores. These constituents occur naturally in all parts of plants such as leaves, stem, and roots. According to WHO, a significant portion (65-80%) of the population still relies on traditional plant-based remedies for the primary healthcare necessities (Shyma *et al.,* 2012). Chemical compounds derived out of plants have wider use in many fields such as agriculture, production of drugs, flavour and fragrances, dyes and pigments, pesticides and food additives. Most of the synthetic drugs that being used in medicines and commercial applications are chemical modifications or copies of naturally obtained substances. The interest to find out more chemical compounds from plants is increasing as a number of infectious agents are becoming more resistant to available antimicrobial compounds. In this context, It is therefore necessary to develop novel drugs derived from plant-based secondary metabolites.

Xanthophyllum arnottianum Wight is one of the 94 species of in the genus *Xanthophyllum* and belongs to the family Polygalaceae. It is a large shrub up to 8-meter tall, grey, smooth bark with orange blaze. These plants occur in evergreen to semi-evergreen forest up to 1200m. It is endemic to Western Ghats. It is distributed in Kerala, Karnataka, Tamil Nadu. As this plant is having restricted distribution and limited to a certain pockets in Southern Western Ghats, seen in Chooralmala region and Ghast section of Thamarassery. However, there exist a limited understanding of this plant's chemical composition that necessitates further investigation. In this context; the aim of this study was focused on the exploration of the chemical constitution of the plant, antioxidant components and antimicrobial properties.

MATERIAL AND METHODS

Plant Sample Collection and Identification

Fresh leaves of the study material - plant *X. arnottianum* Wight was collected from the Chooralmala region of Wayanad district, Kerala – 673 577 and the GPS reading of the location was N-11.5006 °. E76.1581°. The identity of the specimens was confirmed using regional flowering plant floras and also the voucher materials of the specimens were compared. In addition to that further consultations were also held with specimens deposited at MH (Madaras Herbarium situated at BSI, Coimbatore). In addition to that the taxonomists of the institution authenticated the specimen as *X. arnottianum* Wight. The study was conducted at M.S. Swaminathan Research Foundation Community Agrobiodiversity Centre, Wayanad, Kerala, India.

Sample Preparation and Extraction

The fresh leaf sample of about 1 Kg were collected in polythene bags and taken to the laboratory. The leaves were surface sterilized and washed with clean sterile water. Then the leaves were shade dried. After drying the

leaves were powdered using mechanical blender and then transferred into air tight container. For the extraction, methanol was taken as solvent and the procedures for the same was in line with those mentioned by Jigna and Sumitra (2007).

Qualitative Phytochemical Screening

The chemical composition of the extract was determined through various qualitative chemical tests. For phytoconstituents like Alkaloids two tests were conducted, Mayer's Test and Wagner's Test as mentioned by Raaman, 2006, were taken. For the detection of flavonoids, Alkaline reagent test was employed. The method as mentioned by *Shamaila et al.,* 2009, was employed for the detection of tannins and saponins were tested with Foam test. Ferric chloride test was employed to detect phenols and was described by Raaman, 2006. For the detection of proteins (Millon's Test) and carbohydrates (Molish's Test) methodology mentioned by Sadasivam and Manickam., 1991 were used. For Cardiac glycosides- Keller-Killiani test used that was mentioned by De *et al.,* 2010 was adopted.

Quantitative Estimation of Phytochemicals

The leaves of the plant contained certain phytochemicals that were subsequently quantified using various methods. The total carbohydrate present in the sample was tested using Anthrone method as described in the book by Sadasivam and Manickam, 1991. Flavanoid and tannic acid compositions were determined by the adoption of procedures mentioned by Supratim and Anwar, 2008.

Determination of Antioxidants

As the third step of the study, the antioxidant antioxidant capabilities of the leaves of *X. arnottianum* were analyzed. Both enzyme and non-enzymatic antioxidants were studied. The Total Antioxidant capacity was assessed using Phosphomolybdenum method. The enzymatic antioxidants such as Peroxidase were assessed and H_2O_2 Scavenging abilities were also measured using the method mentioned by Supratim *et al.,* 2008.

Screening for the Antibacterial Activity

The antibacterial activity of leaves of *X. arnottianum* Wight was determined against selected cultures of microorganisms kept in the microbial repository of MSSRF Community Agrobiodiversity Centre. The microorganisms collected were *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. Disc diffusion method was used to test the effectiveness of the leaf extract (George *et al*., 2007).

Gas ChromatographyMass Spectrometry (GC-MS)

The Gas chromatography-Mass spectrometry (GCMS) analysis of the sample was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter, and 0.25mm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium (99.99%) was utilized as the carrier gas at a constant flow rate of 1.51ml/min+. Interpretation of mass spectrum of extracts of the lant were conducted using the database of National Institute of Standard and Technology (NIST) library which is having more than 62,000 spectral patterns. The spectrum of the chemical compounds was matched with the spectrum of National Institute of Standard and Technology (NIST) library database. Procedure followed by Sridharan *et al*., 2011 was adopted for this study.

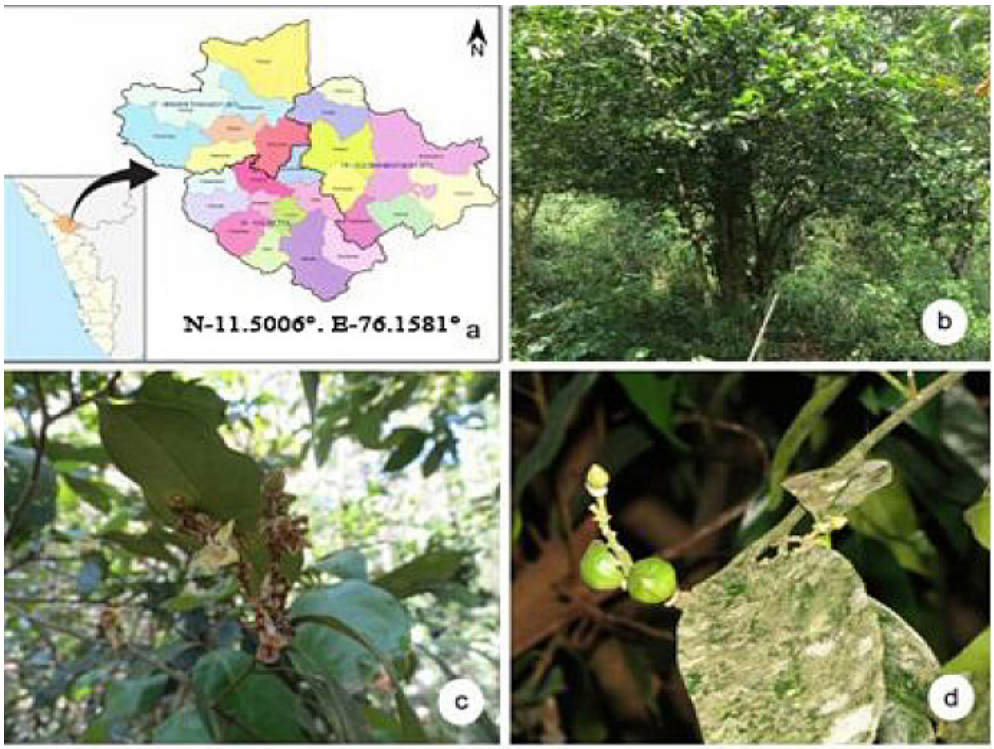

Fig. 1. Map of Kerala with location details and the habitat of the RET plant *X. arnottianum*.

RESULTS AND DISCUSSION

Sample Collection

The fresh leaves of *X. arnottianum* Wight were collected from Chooralmala region of Meppady, Wayanad district and the GPS location details of the place of collection was as N-11.5006 °. E76.1581° as described in the fig.1, where,

a= Map of Kerala Showing the specific location of Wayanad (not to scale). b= The tree *Xanthophyllum arnottianum* Wight,c= Flower of the plant and d= Fruit of the plant.

Table 1. Qualitative determination of the phytochemicals present in the leaves of *X. arnottianum* Wight.

Sl.No.	Phytochemical component	***Xanthophyllumarnottianum*** Wight
1	Carbohydrates	+
2	Protein	-
3	Alkaloid	-
4	Flavonoid	+
5	Tannin	+
6	Saponin	+
7	Phenol	-
8	Glycosides	+

'+' indicate the presence of the phytoconstituent and '-'indicates the absence of the phytoconstituent.

Table 2. Determination of total carbohydrates, Flavonoids and Tannins

Sl. No.	Sample	Quantity (mg/ml^{-1})
1	Total Carbohydrates	0.036±0.0
2	Flavonoids	0.08±0.0
3	Tannins	0.52±0.04

*Mean± standard deviation, n=3.

Qualitative Analysis of Phytochemicals

For the qualitative analysis, sample extracted with solvent methanol of *X. arnottianum* Wight was used. The results showed positive for compounds such as carbohydrates, flavonoids, tannins, saponins, glycosides while protein, alkaloids, phenols gave negative results as given in the table 1.

Quantitative Estimation of Phytochemicals

Methanolic extracts of leaves of *X. arnottianum* Wight showed differences in quantities of phytochemicals present in them.

Determination of Total Carbohydrates

The total carbohydrates content of leaves of *X. arnottianum* was 0.038mg/ml^{-1}. The total carbohydrate content of the sample is shown in Table 2.

The total Flavonoid content of leaves of *X. arnottiaum* was 0.08mg/ml-1 and the tannin content in leaves are measured to be 0.57mg/ml^{-1}. *X.arnottianum* possesses significant levels of constituents like carbohydrates, flavonoid, tannin, saponin and glycosides. At the same time, protein, alkaloids and phenol were absent. The total flavonoid content of leaf of *X. arnottianum* was 0.08mg/ml-1. Similar results were reported in *Polygala arillata* Buch-Ham. ex D. Don leaf extract and their total flavonoid content was reported to be 0.04mg/ml-1 (Radhamani and John, 2016). Both of the plants were included in the family Polygalaceae. The total flavonoid content of leaf extract of *X. arnottianum* was in line with the total flavonoid content of *Polygala arillata.* Flavonoids are chemical compounds reported to have wide range of medicinal properties including anti-viral/bacterial, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging and these compounds have garnered significant scientific interest and are extensively supported by a substantial body of research. To date, the scientific literature documents over 9,000 distinct flavonoids.

In the current investigation the total tannin content of leaf of *X. arnottianum* was 0.52±0.04mg/ml-1. Similar tannin content was reported in *Polygala arillata* Buch- Ham. ex D. Don, leaf extract and their total tannin content was found to be 0.32mg/ml-1. As these two plants are belonging to the same family Polygalaceae tannin distribution have a relation with the family of plants and its distribution. Indian traditional medicines are well reputed for its knowledge on the use of tannin composition in many of its formulations. The flavonoids and tannin composition of these plants could be better utilized for its pharmaceutical and other industrial applications.

GC-MS Analysis

The GC-MS analysis was conducted to identify various compounds present in the ethanolic leaf extract of *X. arnottianum*. Ten principal compounds were identified from the sample. The main compounds were Tetrakis (2,3-Ditert-Butylphenyl)-4,4'-Biphenylene Diphosphonate (47.54%), 2-tert-Butyl-4,6-

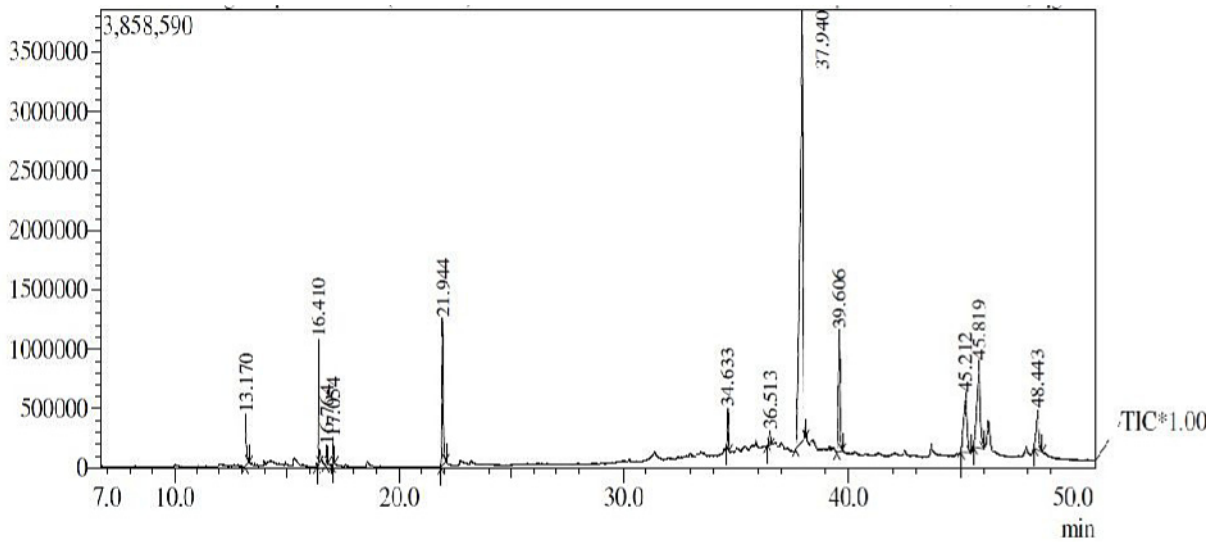

Fig. 2. Chromatogram of the plant extract of GCMS analysis

bis(3,5-di-tert-butyl-4-hydroxybenzyl) Phenol (13.19%), 2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl) Phenol (9.09%), Isophytol, acetate (7.35%), Vitamin E(7.18%). The maximum peak showing compound is Tetrakis(2,3-Ditert-Butylphenyl)-4,4'-Biphenylene Diphosphonate (47.54%). The minimum peak showing compound is 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.65%). The chromatogram was given as Fig. 2. and the major compounds present in the sample was given as table 3.

Ten principal compounds were identified in the ethanolic extract of *X. arnottianum* Wight by GC-MS profiling. The major components are Tetrakis (2,3- Ditert- Butylphenyl)- 4,4-Biphenylene Diphosphonate (47.54%), 2- tert-

Table 3. The major chemical compounds present in the leaf extract (methanol) of *X. arnottianum* Wight

Peak	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	13.170	1499705	2.48	438333	4.46	3,'5'-Dimethoxyacetophenone	180.10
2	16.410	2421680	4.00	1057913	10.77	Neophytadiene	68.05
3	16.764	396647	0.65	159747	1.63	3,7,11,15- Tetramethyl -2-Hexadecen-1-ol	82.05
4	17.054	597666	0.99	228600	2.33	Phytol, Acetate	71.05
5	21.944	4454073	7.35	1235878	12.58	Isophytol, Acetate	71.05
6	34.633	919434	1.52	342050	3.48	Sqalene	69.05
7	36.513	660935	1.09	126938	1.29	Beta- Sitosterol	55.05
8	37.940	28790903	47.54	3633482	36.98	Tetrakis (2,3- Ditert- Butylphenyl)-4, 4'Biphenylene Diphosphonate	57.10
9	39.606	4349973	7.18	1022509	10.41	Vitamin E	165.10
10	45.212	5504834	9.09	501934	5.11	2-Tert-Butyl-4,6-Bis (3,5-Ditert-Butyl-4-HydroxyBenzyl) Phenol	57.10

Butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol (13.19%), 2- Tert- Butyl-4,6- Bis (3,5-Di-Tert-Butyl 4-Hydroxybenzyl) Phenol (9.09%), Isophytol, acetate (7.35%), Vitamin E (7.18%), Neophytadiene (4%), 3,5-Dimethoxyacetophenone (2.48%). Among these compounds 4-Biphenylene Diphosphonate can be used as as an antioxidant. 2- tert- Butyl-4, 6- bis(3,5-di-tert-butyl-4-hydroxybenzyl) phenol can be used as biopesticides and could also be further tested for its pharmacological properties. Isophytol is used in the fragrance industry and used in cosmetics, shampoos, toilet soaps and detergents. The presence of acetate in plant extracts suggests potential applications in various cosmetic and pharmaceutical industries for product processing and preservation. Due to the antioxidant activity of vitamin E in plants have a major role in imparting tolerance to several abiotic stresses. Neophytadiene has good analgesic, antipyretic, anti-inflammatory, antiarthritic and anticancer activities. 3,5-Dimethoxy acetophenone used as anticancer agents. Similar study was reported in *Polygala chinensis* L. Fourteen compounds were identified from ethanolic extract of that plant through GC-MS studies.

Determination of Antioxidants

The leaves of *X. arnottianum* Wight were then taken for testing its antioxidant content. The results obtained are shown in table 4. The total antioxidant capacity was determined. Further investigations were conducted to assess the plant's enzymatic and non-enzymatic antioxidant components, including peroxidase and hydrogen peroxide (H_2O_2) scavenging activity.

The total antioxidant capacity of *X. arnottianum* is 0.28±0.04mg/ml. The peroxidase activity is 0.15±0.03mg/ml-1 and H_2O_2 scavenging activity of *X. arnottianum* is 0.62±0.04mg/ml-1. The results indicated that plant has antioxidant properties.

The total antioxidant capacity of leaf extract of *X. arnottianum* Wight was measured as 0.28±0.04mg/ml-1. Antioxidant potential of this plant demonstrated to possess important as many of the life-threatening diseases (cardio vascular and neuro degenerative) preventing medicines could be developed out of this plant specimen. The Hydrogen peroxide scavenging activity of *X. arnottianum* was 0.62±0.04mg/ml-1. The study also revealed the peroxidase activity of leaf extract of *X. arnottianum* Wight and was measured to be 0.15±0.03mg/ml-1. Peroxidase have a significant role in the reinforcement of cell wall, enhanced production of reactive oxygen species as signal mediators and antimicrobial agents.

Antimicrobial Activity

The antimicrobial efficacy of the plant extract was assessed against three distinct clinical pathogens namely *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* and are gi ven in table 5.

Table 4. Antioxidants Compositionsof the Leaves of *X. arnottianum* Wight

Sl.No.	Test	Quantity (mg/ml^{-1})
1	Total antioxidant capacity	0.28±0.04
2	Peroxidase	0.15±0.03
3	H_2O_2 scavenging activity	0.62±0.04

*Mean± standard deviation, n=3

Table 5. Antimicrobial properties of crude extract of *X. arnottianum* against selected microorganism.

Sl.No	Name of the organism	Zone of inhibition(mm)			
		Plant extract concentration and Inhibition zone in mm			
		25%	50%	75%	100%
1	*Klebsiella pneumoniae*	5.3±0.47	7.3±0.16	7.5±0.41	8.3±0.14
2	*Staphylococcus aureus*	7.2±0.24	9.4±0.47	13.3±0.42	20.4±0.45
3	*Escherichia coli*	5.4±0.43	6.4±0.28	7.6±0.45	7.4±0.46

*Mean± standard deviation, n=3

The plant extract demonstrated the most potent antimicrobial activity against *Staphylococcus aureus*, with subsequent effectiveness against *Klebsiella pneumoniae* and *Escherichia coli*. The measurements were *Staphylococcus aureus* showed 20mm followed by *Klebsiella pneumoniae* with 8mm and least inhibition was to the *Escherichia coli* with 7mm. These maximal inhibition rates were achieved at the highest tested concentration of the plant extract, which was 100 percent.

The ethanolic extract of *X. arnottianum* Wight demonstrated antimicrobial activity against three clinical pathogens that were evaluated. Methanolic extract showed zone of inhibition against *Klebsiella pneumoniae* was found to be 8mm, against *Staphylococcus aureus* was 20mm and against *Escherichia coli* was 7mm. The observed inhibition values were all attained at the highest tested concentration of the plant extract, i.e., 100 percent. Similar study was reported in *Polygala javana* plant in its petroleum extract against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Uthiraselvam *et al*., 2012).

CONCLUSION

The study revealed the chemical constitution, antioxidants and antimicrobial properties of the plant *X. arnottianum* Wight is first of its kind attempted to explore its uses and economic values. The study throws light on many aspects of the composition of the plant especially its composition of flavonoids (0.08±0.0mg/ml-1), tannins (0.52±0.04mg/ml-1) and antioxidants like peroxidase (0.15±0.03 mg/ml) and H_2O_2 scavenging activity of 0.62±0.04mg/ml. The plant extract demonstrated inhibitory effects against all microorganisms subjected to testing in which highest inhibition rate was shown by *Staphylococcus aureus* with 20mm followed by *Klebsiella pneumoniae* with 8mm and *E.coli* with 7mm. Detailed analysis with sophisticated equipment like GCMS reveal that as many as 10 important chemicals of importance was present in this plant in which a few has bio pesticidal properties (6- bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol), antioxidant activities and other compounds of commercial interests.

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