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Characterization of *Plumbago zeylanica* Linn. through GCMS analysis and survey of pharmacological properties of major compounds

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Abstract
Plumbago zeylanica Linn. a medicinally rich plant, used in ayurveda to cure several diseases. The plant was exploited for preliminary analysis and pharmacological activities. The bioactive compounds present crude extracts of *Plumbago zeylanica* showed various pharmacological activites. In this study the extracts of *Plumbago zeylanica* was analyzed for various bioactive compounds responsible for pharmacological effects. The extracts were subjected to GCMS analysis, the area percentage gave the information about the amount of compound present in the extract and properties of major compounds were analyzed by literature survey.

Keywords: plumbago, GCMS, chloroforom, root extract, chitramula

Introduction
Plumbago zeylanica Linn. belongs to Plumbaginaceae is a medicinal plant which is highly valued in ayurveda and folk for the treatment of asthma cough and gastrointestinal disorders**.** Plants have been exploited for several pharmacological activities since ancient times. The drugs presently available have been obtained from natural resources have been the basis of many traditional medicinal systems, they provide new remedies for several diseases to human kind. Phytochemicals are the natural bioactive compounds found in plants that is formed during stress condition. These chemicals are referred to as "secondary metabolites". That includes alkaloid, flavonoids, cucumarins, gums, tennis, terrines, phenols and so on, these phytochemicals are originate in plant food material that works through nutrients and dietary fiber to defend body against diseases.
Plumbago zeylanica occurs throughout the tropics and sub tropics. It possibly originates from south-east Asia, from where it may have been distributed as a medicinal plant and ornamental. it occurs throughout most of tropical Africa. *Plumbago zeylanica* also known as white leadwart, chitrak, chitramula in reginal languages.
Plumbago is native to South East Asia, it is an herb that also grows in India as a wild and has been used by tribal and rural people as a folk medicine in traditional system.
Plant usually grows up to 6 feet, much branched with dark green leaves and creamy white flowers. It flowers all year long but needs full sun to partial shade with intermediate to warm temperature. The fruits of *Plumbago zeylanica* like a small coltbur, soft spines with glue will stick to any thing. *P.zeylanica* can be grown in a variety of soils, such as red laterites soil with very little topsoil to deep black soil.

Materials and Methods
Collection of plant sample from study area
The study area, Bhadravathi, is one of the taluks of Shimoga district. The root of *P. zeylanica* was brought from study area and dried under shade. Powdered sample was used to extract the phytochemicals using Petroleum ether, Chloroform and Methanol. The study area and habit of *Plumbago zeylanica* is shown in fig 1.

Fig 1: The study area and habit of *Plumbago zeylanica*

GC-MS analysis

Gas chromatography Mass spectroscopy (GC-MS), a compatible techniques and most commonly used analysis for identification and quantification purposes. The unknown organic compounds were determined by matching the spectra with reference spectra (Alagammal M *et al*, 2012) [1].

Gas chromatography analysis was carried out at Institute of Analysis of Dairy, Food and Cultures (IADFAC) Bangalore. It is one of the key techniques, generally used for identification of different groups of bioactive compounds.

Gas chromatograph study includes the introduction of sample extract into the GC Column, separation of components on an analytical column, detection of target analysis using Mass Spectrometric (MS) detector (Mohan V R 2011) [6].

Identification of components

Identification of bioactive compounds was based on the molecular mass, molecular structure and calculated fragments. The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns are used for interpretation of mass spectra. The name, molecular weight, molecular formula and structure of the components of the samples were determined. By comparing its average peak area to the total areas, the relative percentage amount of every compound was calculated. The spectrum of the unknown compound was compared with the spectrum of the compound stored in the NIST library, to determine whether the plant species consists any compound which have potential drug content and prove its traditional use as an herbal medicine (Thomas E *et al*, 2003) [9].

Result

Gas chromatography-Mass spectroscopy (GC-MS) Analysis

GC-MS analysis of Petroleum ether extract of Root of *P. zeylanica*

GC-MS of root of *Plumbago zeylanica* Petroleum ether extract revealed the presence of 19 components which are listed in the table 1. out of which structure and activity of 5 major compounds are in the table 2. The Chromatogram of Petroleum ether extract of root of *P. zeylanica* is shown in chart 1.

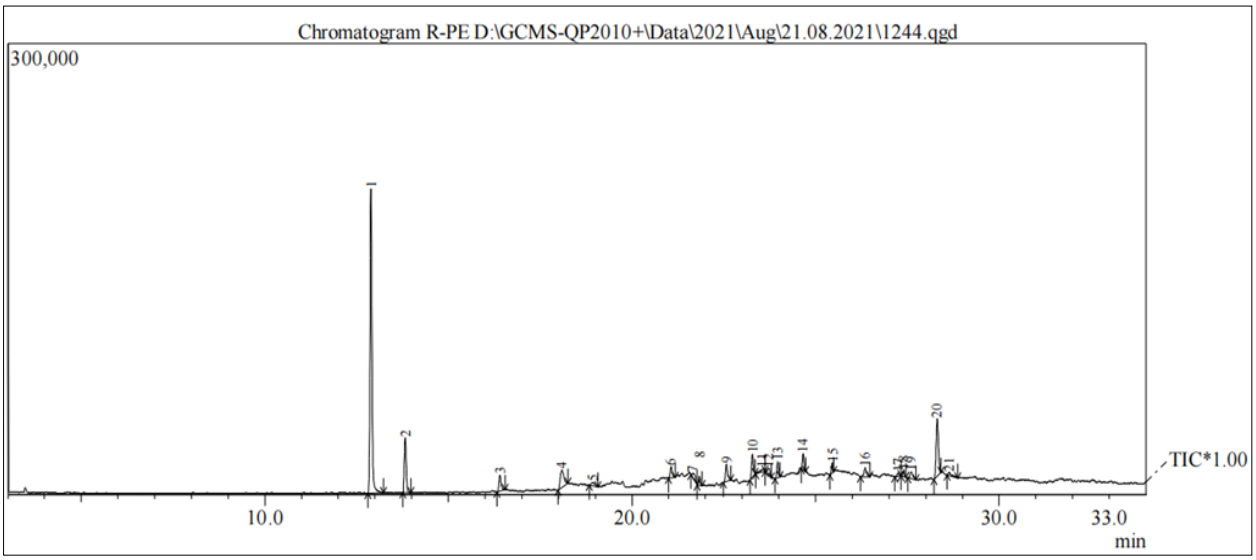
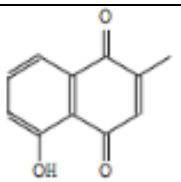
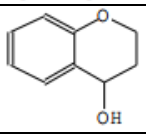
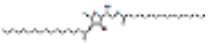
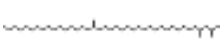


Chart 1: Chromatogram of Petroleum ether root extract of *P. zeylanica*

Table 1: GC-MS analysis of Petroleum ether extract of root of *P. zeylanica*

Peak	Retention time	Peak area %	Compound name	Molecular formula	Molecular weight	Chemical structure
1	12.893	48.97	1,4-Naphthalenedione, 5-hydroxy-2-methy-	C11H803	188	
2	13.827	8.26	4-Chromanol	C9H10O2	150	
3	16.401	2.72	1-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H6808	652	
4	18.093	5.17	Z,Z-8,10-Hexadecacadien-1-ol	C16H30O	238	
5	18.915	0.88	Tetracontane,3,5,24-trimethyl	C43H88	604	

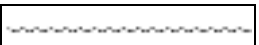
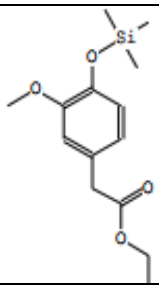
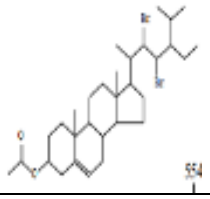
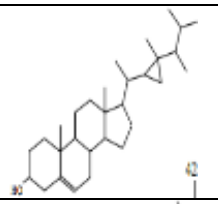
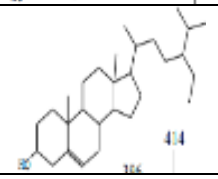
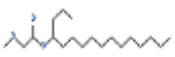
6	21.068	1.62	Pentatriacontane	C35H72	492	
7	22.572	2.91	Tetratetracontane	C44H90	618	
8	23.284	3.26	Hexatriacontane	C36H74	506	
9	23.564	0.98	Tetracosa-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23-hexamethyl-	C30H52O2	444	
10	23.724	1.42	Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl ester	C14H22O4Si	282	
11	23.967	2.08	Hexatriacontane	:C36H74	506	
12	24.665	2.09	Heptacosane	C27H56	380	
13	25.452	1.36	Dotriacontane	C32H66	450	
14	26.349	1.94	Nonacosane	C29H60	408	
15	27.241	0.62	22,23-Dibromostigmasterol acetate	C31H50Br2O2	612	
16	27.399	0.86	Nonacosane	C29H60	408	
17	27.604	1.42	Gorgost-5-en-3-ol, (3. beta.)-	C30H50O	426	
18	28.317	10.10	Beta, -sitosterol	C29H50O	414	
19	28.637	0.94	Methoxyacetic acid, 4-hexadecyl ester	C19H38O3	314	

Table 2: Pharmacological properties of major compounds of Petroleum ether extracts of root of *P.zeylanica*

SI. No	Compound Name	Peak Area %	Biological Activity
01	1,4-Naphthalenedione, 5-hydroxy-2-methy-	48.97	Anticancer activity (Devi *et al*., 1994). Macrofilarici-dal activity (Mathew *et al*., 2002)(Rao C V etal,1998)
02	4-Chromanol	8.26	Antibacterial activity (Quis *et al*.,2021)
03	Z,Z-8,10-Hexadecacadien-1-ol	5.17	antifungal activities (Devi *et al*.,1994)
04	Beta,-sitosterol	10.10	Anti-inflammatory and cytotoxic effects (Arunachalam *et al*.,2021)
05	Hexatriacontane	3.26	Antibacterial activity (Swathi *et al*.,2019)

GC-MS analysis of Chloroform extract of Root extract of *P. zeylanica*

GC-MS of Plumbago *zeylanica* extract of Chloroform sample revealed the presence of 19 components having different pharmacological importance are listed in the table 3. Out of which structure and activity of 6 major compounds are in the table 4. The Chromatogram of Petroleum ether root extract of *P. zeylanica* is shown in chart 2.

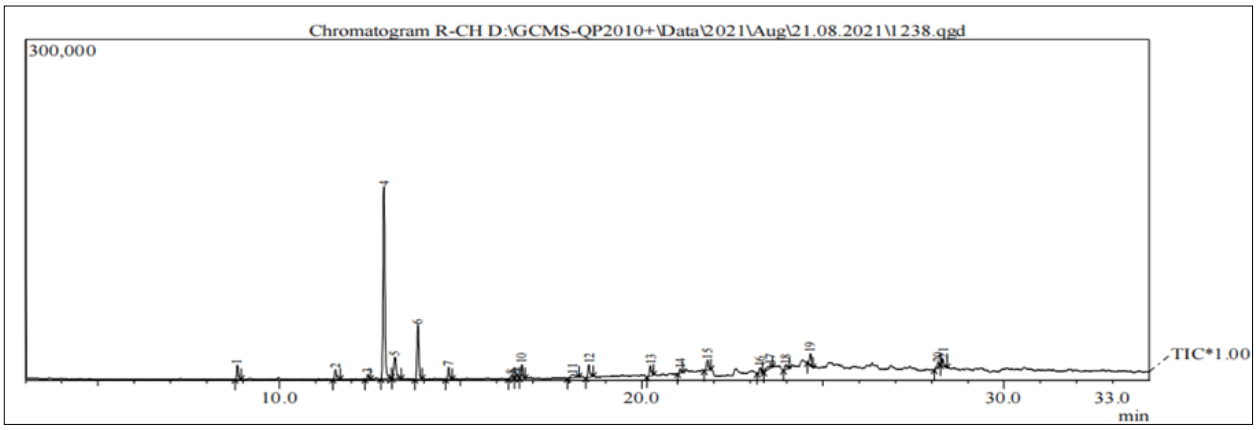
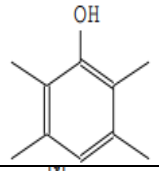
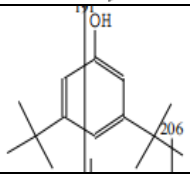
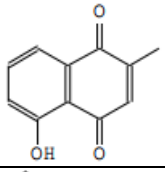
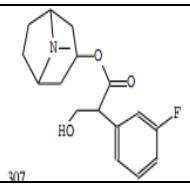
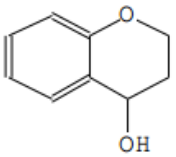


Chart 2: Chromatogram of Petroleum ether root extract of *P. zeylanica.*

Table 3: GC-MS analysis of Chloroform extract of root of *P. zeylanica*

Peak	Retention time	Peak % Area	Compound name	Molecular formula	Molecular weight	Chemical structure
1	8.852	3.04	Phenol, 2,3,5,6-tetramethyl-	C10H14O	150	
2	11.554	1.98	Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22O	206	
3	12.446	1.04	1-Tridecene	C13H26	182	
4	12.891	46.80	1,4-Naphthalenedione, 5-hydroxy-2-methyl-	C11H8O3	188	
5	13.194	6.41	Fluoroatropine	C17H22FNO3	307	
6	13.832	12.43	4-Chromanol	C9H10O2	150	

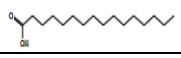
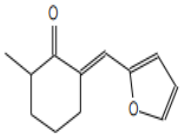
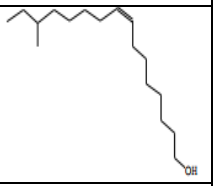
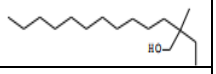
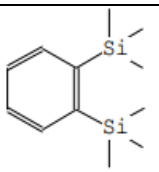
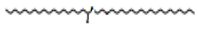
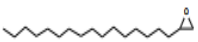
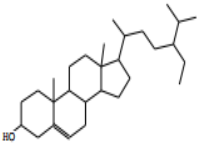
7	14.675	2.47	1-Hexadecanol	C16H34O	242	
8	16.399	0.77	n-Hexadecanoic acid	C16H32O2	256	
9	16.559	0.97	Cyclohexanone, 2-(2-furanylmethylene)-6-methyl-	C12H14O2	190	
10	16.697	2.79	1-Nonadecene	C19H38	:266	
11	18.098	1.88	®-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	C17H34O	254	
12	21.067	0.84	Tridecanol, 2-ethyl-2-methyl-	C16H34O	242	
13	21.815	2.84	1-Hentetracontanol	C41H84O	592	
14	23.273	1.37	Tetrapentacontane, 1,54-dibromo-	C54H108Br2	914	
15	23.559	1.96	1,2-Bis(trimethylsilyl)benzene	C12H22Si2	222	
16	23.966	1.24	Tetracosane	C24H50	338	
17	24.662	2.56	Octadecanoic acid, 2-(octadecyloxy)ethyl este	C38H76O3	580	
18	28.168	1.16	Oxirane, hexadecyl-	C18H36O	268	
19	28.307	1.70	beta.-Sitostero	C29H50O	414	

Table 4: Pharmacological properties of major compounds of Chloroform extracts of root of *P.zeylanica*

SI. No	Compound Name	Peak Area %	Biological Activity
01	1,4-Naphthalenedione, 5-hydroxy-2-methyl-	46.80	Anticancer activity (Devi *et al*., 1994). Macrofilarici-dal activity (Mathew *et al*., 2002)
02	Fluoroatropine	6.41	Synthesis and biological activity of fluorescent nenicotinoid insecticide thiamethoxam (Taillebois *et al*., 2014)
03	4-Chromanol	12.43	Antibacterial activity and antibiofilm activity (Teanpaisan *et al*.,2017)
04	1-Hexadecanol	2.47	Antibacterial activity (Kubo *et al*.,1993) & Antimicrobial activity (Susanti *et al*.,2013)
05	1-Hentetracontanol	2.84	Antimicrobial activity (Sarmad *et al*.,2012)
06	Octadecanoic acid, 2-(octadecyloxy)ethyl este	2.56	Antimicrobial activity (Jasim *et al*.,2015)

GC-MS analysis of Methanol extract of Root extract of *P. zeylanica*

GC-MS of Plumbago *zeylanica* extract of Methanol sample revealed the presence of 12 components having different pharmacological importance are listed in the table 5. out of which structure and activity of 7 major compounds are in the table 6. The Chromatogram of methanol root extract of *P. zeylanica* is shown in Chart 3.

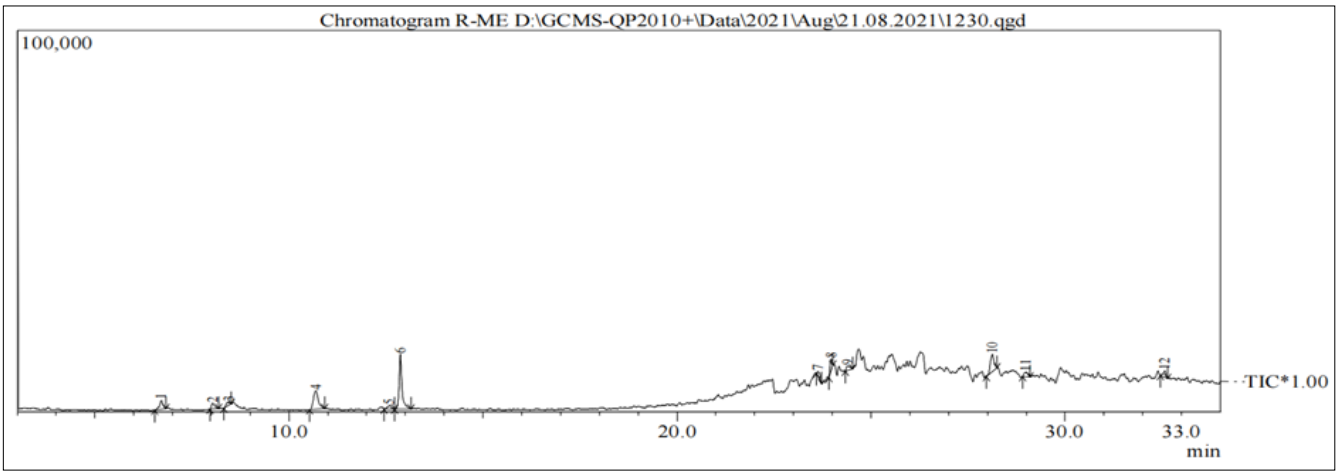
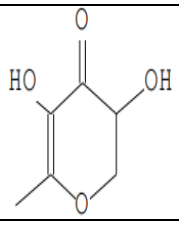
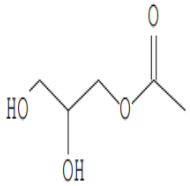
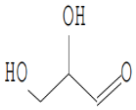
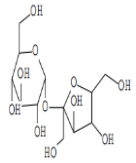
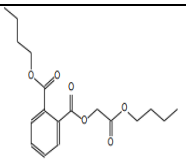
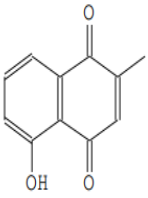
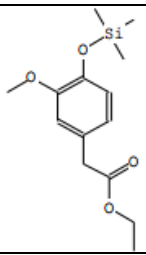


Chart 3: Chromatogram of methanol extract of root of *P. zeylanica*

Table 5: GC-MS analysis of Methanol extract of root of *P. zeylanica*

Peak	Retention time	% Area	Compound name	Molecular formula	Molecular weight	Molecular structure
1	6.713	5.99	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methy	C6H8O4	144	
2	8.035	3.86	1,2,3-Propanetriol, monoacetate	C5H10O4	134	
3	8.436	2.65	Propanal, 2,3-dihydroxy-, (S)- $$ 2,3-Dihydroxypropanal	C3H6O3	90	
4	10.694	18.68	Sucrose	C12H22O11	342	
5	12.591	2.63	Phthalic acid, butyl ester, ester with butyl glycolate	C18H24O6	336	
6	12.872	33.76	1,4-Naphthalenedione, 5-hydroxy-2-methyl-	C11H8O3	188	
7	23.623	3.11	Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl este	C14H22O4Si	282	

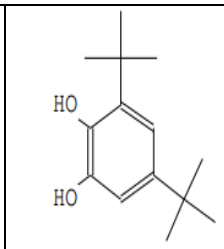
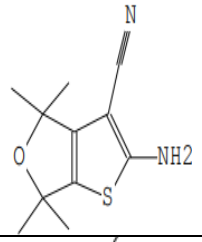
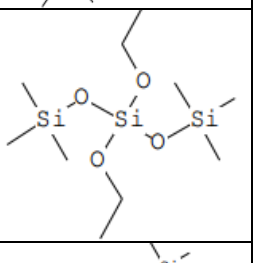
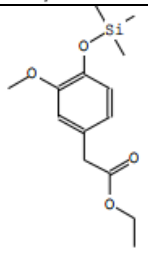
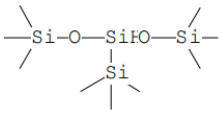
8	23.968	5.04	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	C14H22O2	222	
9	24.386	2.97	2-Amino-4,4,6,6-tetramethyl-4,6-dihydro-thieno[2,3-c]furan-3-carbonitrile	C11H14N2OS	222	
10	28.118	13.89	Silicic acid, diethyl bis(trimethylsilyl) ester	C10H28O4Si3	296	
11	28.993	3.69	Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl ester	C14H22O4Si	282	
12	32.564	3.71	Trimethylsilyl-di(timethylsiloxy)-silane	C9H28O2Si4	280	

Table 6: Pharmacological properties of major compounds of Methanol extracts of root of *P.zeylanica*

Si. No	Compound Name	Peak Area %	Biological Activity
01	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methy	5.99	total flavonoids - (Teoh *et al*.,2014)
02	Sucrose	18.68	Physico-chemical studies of sucrose thin films (Predoi *et al*.,2010)
03	1,4-Naphthalenedione, 5-hydroxy-2-methyl-	33.76	Macrofilaricidal activity (Mathew *et al*.,2002)
04	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	5.04	antioxidant and antimicrobial activity (Nyaberi *et al*.,2017)
05	Silicic acid, diethyl bis(trimethylsilyl) ester	13.89	Anti-inflammatory, Antioxidant, Antibacterial effect (Kumar *et al*.,2002)
06	Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl	3.69	Antimicrobial and antioxidant activity (Ertürk *et al*.,2016)
07	Trimethylsilyl-di(timethylsiloxy)-silane	3.71	antibacterial, antifungal and antioxidant activities (Ceren Yavuz *et al*.,2016)

Conclusion

The present investigation revealed the medicinal uses of its phytochemicals and pharmacological properties of *Plumbago zeylanica* L. The retrieved data documented that *Plumbago zeylanica L* is a good source of phytoconstituents and has excellent therapeutic properties. The major constituents reported in the plant were alkaloids, flavonoids, saponins, phenolic compounds, steroids, triterpenoids, tannins, and glycosides. Literature survey indicates its huge utility towards variety of diseases such as cough, ulcer, cardiovascular disorders, asthama, liver problems, cancer diabetes, wound healing, obesity etc. This study proved the traditional claims on its health benefits. Most of the work has been done on crude extracts according to literature survey therefore, there is need for future research to isolate and characterize pharmacologically potent molecule that have extensive medicinal properties in *Plumbago zeylanica* L. Elucidation of the structure of these agents, scientifically validate the presence of active compound and help in comparison of similar compound. Isolation of these compounds and utilizing them as a drug from plant sources reduces the side effects.

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