

Phytochemistry and Pharmacology of *Iris kashmiriana*

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ABSTRACT

Iris kashmiriana belonging to the family Iridaceae, is found commonly in graveyards in the Kashmir region of India, as well as in some areas of Pakistan. This plant is used as a traditional medicinal plant by different local communities. Ethnopharmacological studies have revealed the presence of isoflavones like isonigrin (1) which is a new isoflavone and isoirisolidone (2) and Irisolidone (3) which were isolated first time from a natural source. This emphasis of this review is to focus on traditional uses, pharmacological uses, as well as phytoconstituents present in this species. But as this species is critically endangered also. By exploring more about this species, it might help in getting this species in the least concern or prevent it from being extinct. Scientific investigation has found that this plant has a lot of pharmacological activities like antimicrobial, antitumor, anthelmintic, immunomodulatory, antiseptic, antioxidant, and also used in cold, flu, malaria, joint pain, anti-inflammation, toothache, etc. This plant contains several constituents, including isoflavones, with pharmacological activities some of them are still not been fully explored yet. In this review, an approach was made to study ethnopharmacology, phytochemistry, and pharmacological investigations, of *Iris kashmiriana*, which can be explored more, so that people could know more about this plant, and start growing this medicinal plant.

Keywords: *Iris kashmiriana*, Kashmir, Traditional plant, Endangered plant, Antitumor, Immunomodulator.

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INTRODUCTION

Iris kashmiriana (Figure 1) belongs to the family Iridaceae, which is commonly known as 'Mazarmund' and 'Kabriposh' in the Kashmir region of India. 'Mazarmund' in local language means 'mazar' means graveyard or cemetery, mond means root or rhizome.^[1] *Kashmiriana* is due to the origin of the plant from Kashmir. The genus *Iris* which belongs to the family Iridaceae contains around 300 species, of which 12 species are found in India.^[2] Nature has given us a lot of treasures, in the form of medicinal plants. In ancient times, people used only these plants to get cures for diseases and even used them today in most developing counties.^[3] The first pure natural product isolated from an herbal source was morphine.^[4] As herbal medicines have great demand because of their efficacy, safety, and lesser side effects.^[5] There are huge areas in the research and development of this plant, as this plant contains isoflavones like isonigrin (1) which is a new isoflavone, whereas isoirisolidone (2) and Irisolidone (3) are also found in this plant, which was isolated first time from a natural source.^[6] This medicinal plant is yet to be explored and it's rare also. This species is critically endangered, if necessary, actions are not taken at the appropriate time, this medicinal plant will get extinct.^[7]

Classification^[8]

Kingdom- Plantae; **Phylum-** Angiosperms; **Class-** Monocots; **Order-** Asparagales; **Family-** Iridaceae; **Genus-** *Iris*; **Species-** *Iris kashmiriana*.

Morphology^[9]

The *Kabriposh* is derived from 'Kabri' and 'posh', Kabri means grave and posh means flower, the other name *Mazarmund*, where 'Mazar' means grave and 'mund' means root. It is a medicinal plant found normally on the graves in the Kashmir region. Flowers are white or violet and occur between April-June.^[9] Altitude range 1600-2200 m from sea level. Aerial stem 50-100 cm tall, with 1,2 branches and each branch contains 2-3 flowers. Perianth fragrant, white with blue markings, and yellow-green veins, floral tube 2-2.5 cm long, falls 6-10 cm long. Ovary green with ridges and grooves, Stigma entire, Filament 1-2 cm long, Anther 1.5 cm long, white, Rhizome is thick and stout, Leaves are 4-6 in number and up to 60 cm in length, 3-5 cm broad, straight, glaucous, scarios margin. Seeds globular, red-brown, wrinkled. Chromosome number: 2n = 44.

Distribution

It is native to topical Asia, found in India, Nepal, Pakistan, Afghanistan.^[10] *Iris kashmiriana* found

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Figure 1: *Iris kashmiriana*.

mainly in Kashmir region at an altitude of 1600-2200 m from sea level. It can also be obtained from wild accessions that were growing in Parvati valley, in district Kullu, Himachal Pradesh, in India, at an altitude of 3500-4000 m from sea level.^[11]

Traditional uses

Antiseptic

It is used as traditional method of treatment or folk method of treatment in local Bakarwal community of Jammu Kashmir region of India. Flowers from the plant are used as antiseptic.^[1]

Joint Pain

Iris kashmiriana is used in powder form to treat joint pains as a traditional system of medicine in the Kajinaag range of Kashmir Himalaya. In the Bandipora area, which is also in Kashmir (India), the dried rhizome part of the plant is used to cure joint pains.^[12]

Milk Production in Animals

A mixture of *Iris kashmiriana* and Jaggery (Gur) was grinded and given to animals to increase their milk production and to overcome general body weakness.^[13]

Phytoconstituents

These are the compounds present in the plant, which are responsible for the physical, chemical, and other properties of the plant. Which may show medicinal properties. Like saponins, tannins and phenolic compounds, glycosides, carbohydrates, phenolics, antioxidants, flavonoids, terpenoids, triterpenes, amino acids and proteins, glucose and isoflavons. The primary phytoconstituents are enlisted in Table 1.

The Isoflavones present in *Iris kashmiriana* are (Figure 2)-irisolidone 7-O- α -D-glucoside, irigenin (4), irilone (5), iriflogenin, and iriskashmirianin (6).^[15] 4'-hydroxy-8-methoxy-6,7-methylenedioxyisoflavone (isonigrin) (1), 5,6-dihydroxy 4',7-dimethoxyisoflavone (isoirisolidone) (2), and 5,7-dihydroxy -4',6-dimethoxyisoflavone (irisolidone) (3), where isonigrin (1) is a new

Table 1: Preliminary phytochemical analysis of *Iris kashmiriana*.^[10,14]

Phytochemicals	Present/ Absent
Alkaloids	-
Carbohydrates	+
Tannins	+
Flavonoids	+
Phenols	-
Phytosterols	+
Proteins	+
Saponins	+
Diterpenes	+
Glycosides	-

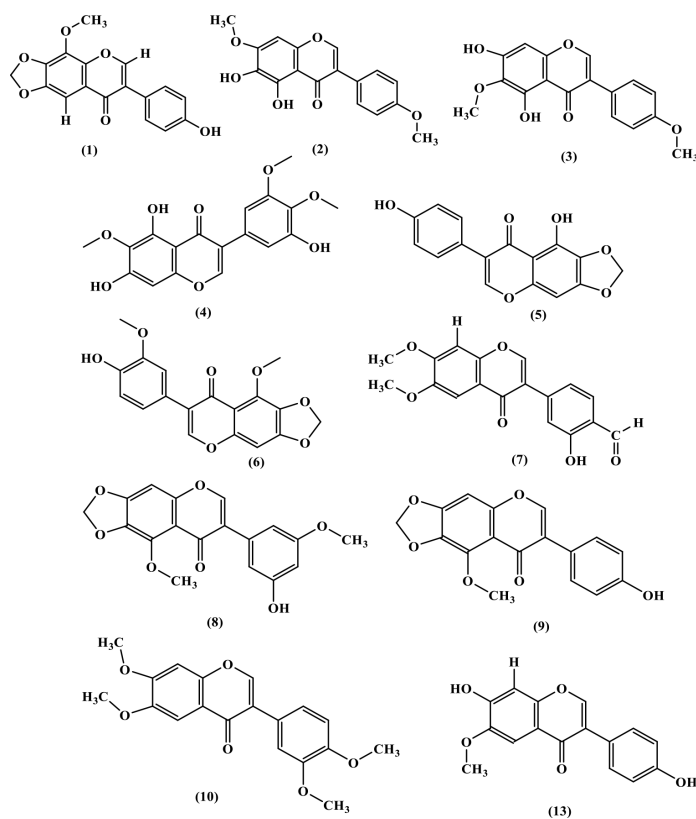


Figure 2: Isolated Isoflavones from *Iris kashmiriana*.

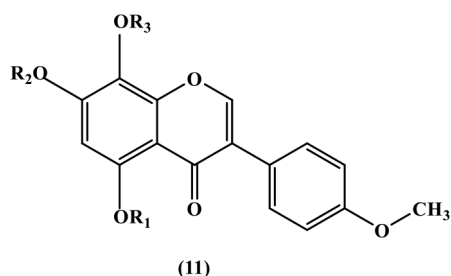
isoflavone and isoirisolidone (2), is reported to be first time isolated from a natural source.^[6] Isocladrastin (7), 13'-hydroxy 6,7,4'-trimethoxy-I, Kashmirioenin I 4'-hydroxy-3',5' dimethoxy-6,7-methylenedioxy-I.^[16] 7-O-b-D-Glu-5, 8-dihydroxy-4'-methoxyisoflavone, aglycone, 5,7,8-trihydroxy-4'-methoxyisoflavone, 5,7,8-triacetate isoflavone, 7-hydroxy-5,8,4'-tri-methoxy isoflavone, 7-hydroxy-5,8,4'-trimethoxy isoflavone, 7-acetyl-5,8,4'-tri-methoxy isoflavone, 7-hydroxy-6-acetyl-4'-methoxy isoflavone.^[11] On chemical re-investigation, other compounds from the rhizome were isolated Isoiriskashmirianin (8) and Irisolone (9)^[17] and presence of Irisolone methyl ether (10) was also observed.^[18] The chemical structures of these compounds are enlisted in Table 2.

On fractionating the methanolic extract in n-butanol, resulted in isolation of 2 glycosides.^[11]

Table 2: Biological activities of Plant extracts.

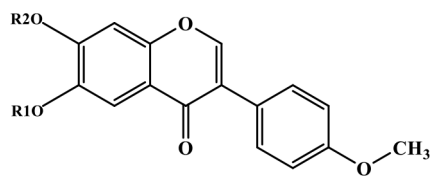
Sl. No.	Part used	Solvent used for extraction	Animal	Test performed	Result	References
2.1	Rhizome	Methanol	Albino mice	Acetic acid induced writhing test Hot Plate test	Decrease in writhing but comparatively higher than control Show dose-dependent Antinociceptive effect	[14]
2.2	Rhizome	Methanol, Water	Sheep	Acute oral toxicity <i>In vitro</i> anthelmintic activity <i>In vivo</i> anthelmintic activity	No mortality till 2000 mg/ Kg Crude aqueous extracts of <i>I. kashmiriana</i> exhibited greater anthelmintic activity against <i>H. contortus</i> than crude methanolic extract (P < 0.05). The mean Eggs per gram count in faecus show significantly reduced.	[23]
2.3	Rhizome	Methanol	Cell lines	Cancer	Shows anti-proliferative activity against selected cell lines and targeting NF-kappaB.	[11]
2.4	Rhizome	Methanol, Water	-----	Preliminary phytochemical screening	-----	[10]
2.5	Leaves	Ethanol, Water	Sabouraud Dextrose Agar homogenized Petri dish	Antifungal activity	Show dose-dependent Antifungal effect, and zone of inhibition was more in aqueous as compare to ethanolic	[20]
2.6	Rhizome	Chloroform, Ethyl acetate, Methanol	Different microbes	Antimicrobial activity	Different extracts possess antimicrobial effect, but results were lower than standard reference	[19]
2.7	Leaves	Ethanol	-----	<i>In vitro</i> antioxidant activity, total phenolic and flavonoid content	It contains potential antioxidant activity	[25]

➤ **Glycoside 1** elemental analysis found the presence of these compounds^[11]



Sl. No.	R1	R2	R3	Nomenclature
11.1	H	Glu	H	(7-((2S,3S,4R,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy)-5,8-dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
11.2	OCOCH ₃	Glu	OCHOCH ₃	(7-((2S,3S,4R,5S)-tetrahydro-3,4,5-triacetoxy-6-(acetoxymethyl)-2H-pyran-2-yloxy)-5,8-diacetoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
11.3	H	H	H	(5,7,8-trihydroxy-3-(4-methoxyphenyl)-4Hchromen-4-one)
11.4	OCOCH ₃	OCOCH ₃	OCOCH ₃	(5,7,8-triacetoxyoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
11.5	OCH ₃	Glu	OCH ₃	(7-((2S,3S,4R,5S)-tetrahydro-3,4,5-trimethoxy-6-(methoxymethyl)-2H-pyran-2-yloxy)-5,8-dimethoxyoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
11.6	OCH ₃	OH	OCH ₃	(7-hydroxy- 5,8-dimethoxy-3-(4-methoxyphenyl)-4Hchromen-4-one)
11.7	OCH ₃	OCOCH ₃	OCH ₃	(5,8-dimethoxy-3-(4-methoxyphenyl)-4-oxo4H-chrome-7yl acetate)
11.8	H	H	H	(5,7,8-trihydroxy-3-(4-hydroxyphenyl)-4Hchromen-4-one)

➤ Glycoside 2 elemental analysis found the presence of these compounds^[11]



Sl. No.	R1	R2	Nomenclature
12.1	H	Glu	(7-((2S,3S,4R,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yloxy)-6-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
12.2	COCH ₃	H	(7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-6-yl acetate)
12.3	COCH ₃	COCH ₃	(6,7-diacetoxyoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
12.4	H	H	(6,7-dihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
12.5	CH ₃	Glu	(7-((2S,3S,4R,5S)-tetrahydro-3,4,5-trimethoxy-6-(methoxymethyl)-2H-pyran-2-yloxy)-6-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
12.6	CH ₃	H	(7-hydroxy-6-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one)
12.7	CH ₃	OCOCH ₃	(6-methoxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-yl acetate)

Table 3: List of compounds isolated from *iris kashmiriana* with their pharmacological activities.

Compounds	Pharmacological activities	References
Aglycone	Cardiac activity	[26]
Iriflogenin	Cancer chemopreventive	[15]
Kashmigenin	Antimicrobial	[16]
Isonigracin (1)	Immunosuppressant, antimicrobial	[6,24]
Isoirisolidone (2)	Immunostimulating	[24]
Irisolidone (3)	Cardioprotective, anti-viral	[27]
Irigenin (4)	Antimicrobial	[28]
Irilone (5)	Increase progesteron level	[29]
Iriskashmirianin (6)	Anti-tumor	[15]
Isocladrastin (7)	Anti-bacterial, anti-viral	[12]
Tectorigenin (13)	Analgesic, anti-inflammatory	[30]

Pharmacological activity

These pharmacological activities mentioned below are already being proven and available in the literature and activities of isolated compounds are mentioned in Table 3.

Antimicrobial activity

Infectious diseases are the second leading cause of death worldwide. In a study, where methanolic extract was used with different doses of 50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml extract of Plant showed dose dependent Anti-microbial activity, on various micro-organisms like *B. cereus*, *P. auregenosa*, *P. vulgaris*, *E. coli*, *C. albicans*, and *A. niger*. It showed a positive response.^[19] In another study the pre-treatment of *Iris kashmiriana* extract protected the mice against mortality due to the induced *E. coli* sepsis and this is compared with standard.^[19] In other study aqueous extract showed maximum antimycotic activity, against penicillium expansum, *Rhizoctonia solani*. Moderate antifungal activity was observed against *Mucor plumbeus* and *Trichothecium roseum*.^[20]

Anti-tumor

Cancer is known to be the most complex and challenging disease known to mankind and concerns public health. Its current treatment is far from being satisfactory, its efficacy is limited and serious side effects are associated with it, and some are even life threatening. It is estimated that the number of new cases of cancer would rise to 70% over the next 2 decades.^[21] Extract of *Iris kashmiriana* showed selective anti-proliferative activity, against epithelial cancer cell lines. This extract has anti-oxidant properties with anti-carcinogenic potential, and suggests a potential role in anti-cancer treatments in future. In this the methanolic extract was used on human cell lines A549 (lung adenocarcinoma), caco-2 (colon adenocarcinoma) as well as mouse non-neoplastic fibroblast cell line NIH-3T3. It showed a significant inhibition of growth in adenocarcinoma cell line (A549) and colon adenocarcinoma (caco-2) in a dose dependent manner, but there was less pronounced effect on mouse-fibroblast cell line (NIH-3T3), it determines cell-type specific effect of the extract. The high radical scavenging activity, determine the presence of potent anti-oxidant compounds, whereas HPLC analysis determines presence of two active compounds – irigenin (4), and Tectorigenin (13).^[22] Besides from other compounds present 5,7,8-trihydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one, 5,7,8-trihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one, 6,7-diacetoxyoxy-3-(4-methoxyphenyl)-4H-chromen-4-one and 5,7,8-triacetoxyoxy-3-(4-methoxyphenyl)-4H-chromen-4-one, showed distinct broad-spectrum anticancer activity. Cell cycle analysis indicates that these compounds induced cell cycle arrest at G2/M phase.^[11]

Anthelmintic Activity

Methanolic and aqueous extract were tested against gastrointestinal nematodes of sheep. Worm motility inhibition assay was used *in-vitro* study whereas *in-vivo* faecal egg count reduction assay was used. The aqueous extract resulted in 85 % worm motility inhibition, whereas the methanolic extracts resulted in 100 % worm motility inhibition. The *in-vivo* anthelmintic activity of aqueous and methanolic extracts in sheep naturally infected with mixed gastrointestinal nematodes species. It also indicated that the aqueous extract exhibits the greater anthelmintic activity under both *in-vivo* and *in-vitro* conditions. It has potency to

contribute to the control of gastrointestinal nematode parasites of small ruminants.^[23]

Immunomodulatory activity

This plant containing a isoflavone which is a new isoflavone 4'-hydroxy-8-methoxy-6,7-methylenedioxyisoflavone (isonigracin) (1), which is first time discovered in this plant. It was isolated from the rhizome of *Iris kashmiriana*. Flowcytometric method was used to study the effect of the production of T-lymphocytes (CD4+ and CD8+ T-cells) and T-cell cytokines (IL-2, IL-4 and IFN- γ) in a dose-dependent manner. The isonigracin (1) showed immunosuppressant activity on T-cells and cytokines. Isoirisolidone (2), other isoflavone isolated from this species, acted as immunostimulatory for both cells and cytokines, whereas their methylated product exhibit stimulatory activity on interleukins, under study and suppressive effect on the production of T-lymphocytes and IFN- γ . Whereas the LD₅₀ in mice was observed to be more than 200 mg/kg body weight. Such a difference in the activity can be due to the positions and manifestation of the positions and nature of substituents present on iso-flavonoid skeleton and affect the potential of these isolated compounds as immunomodulatory agents.^[24]

Antioxidant activity

Plant extract have potential antioxidant activity by donating hydrogen to DPPH (deep violet color) free radical and convert it into α, α -diphenyl- β -picrylhydrazine, which is colorless. The antioxidant potential is indicated by discoloration. Antioxidant activity is directly related with the number of phenols present in extract. It has been indicated that phenolic compounds are directly involved in the scavenging activity of free radicals.

H₂O₂ inactivates various enzymes by oxidizing their thiol (-SH) group. Plant extract showed dose dependent scavenging activity of H₂O₂.

Ferric reducing power showed the extract acts as a good reductant as the extract acts as a good reductant as the extract reduces ferric ion of ferric cyanide to ferrous ion, (Perl's Prussian blue). These directly indicates the plant extract has significant and potential antioxidant activity.^[25]

Others

The plant has been widely used in the traditional system medicine in local area and also in modern clinical preparations to treat cold, flu, malaria, joint pain, toothache, cancer, bacterial and viral, infections and bruise.^[14]

These are most common compounds which are present in the plant, but sometimes according to the climate and weather, it can vary. The phytoconstituents of the plant are mainly responsible for the activity of the plant. Some of these compounds are also found in other plants.

Limitations

As this plant grows in specific habitat at specific altitudes, mainly in Kashmir, from where it also got its name – *Iris kashmiriana*. In Kashmir region, it is found normally in graveyards, near the graves and sometimes over the graves also. Due to ethical issues people don't uproot this plant from graves. Normal people don't even prefer to visit graveyards. Mostly people don't grow this plant at home also because it does not have any economic benefits. And this species comes under critically endangered species.^[7] These are few limitations associated with this plant.^[31]

CONCLUSION

As diseases are getting modified, so effective treatments should also be updated with time. As herbal drugs have lesser side-effects, and it is estimated that 80% of the world's population living in developing

countries uses herbal medicines as their primary source of treatment. As this plant is obtained from the graveyards of Kashmir, due to ethical issues, normally people don't prefer to go there. This might be the reason; this plant is yet to be explored. Traditionally this plant was used in joint pain, antiseptic, and to increase in milk production of animals. This medicinal plant has a lot of pharmacological effects also like anti-tumor, anti-microbial, anthelmintic, immunomodulatory, etc. There are few compounds which were isolated first time from this plant. But it is rare species, and it is also enlisted in endangered species. So, we think people should cultivate this species, so that other medicinal properties could also be identified.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

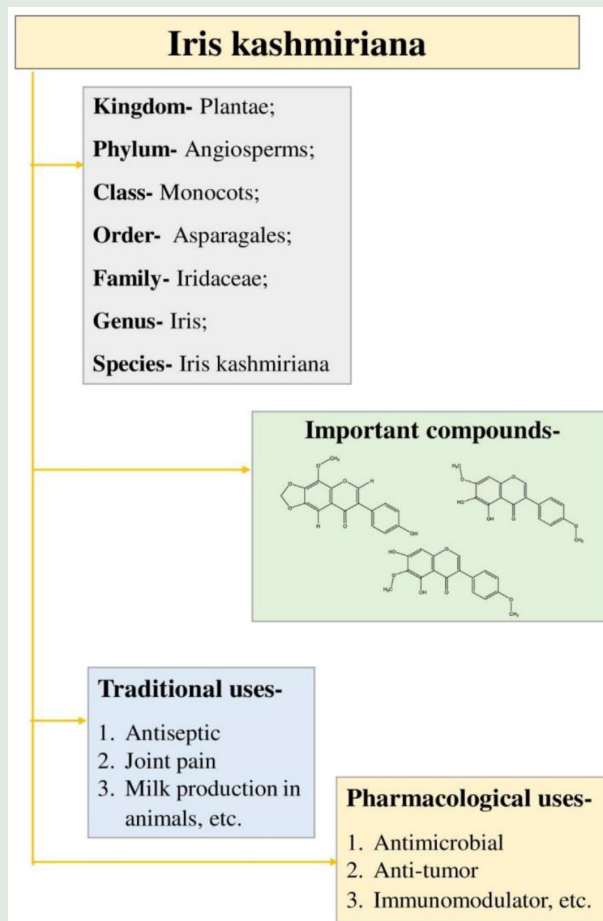
B. cereua: *Bacillus cereus*; **P. auregenosa:** *Pseudomonas aeruginosa*; **P. vulgaris:** *Proteus vulgaris*; **E. coli:** *Escherichia coli*; **C. albicans:** *Candida albicans*; **A. niger:** *Aspergillus niger*; **H. contortus:** *Haemonchus contortus*; **H₂O₂:** Hydrogen peroxide; **NF-kappaB:** Nuclear factor Kappa B; **IFN- γ :** Interferon gamma; **LD₅₀:** Lethal dose 50; **IL-2:** Interleukin 2; **IL-4:** Interleukin 4; **CD4:** Clusters of differentiation 4;

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GRAPHICAL ABSTRACT



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