

## Comparative analysis of the carbohydrate and glycoside contents in bark extracts from *Acacia catechu*

Archana Tiwari <sup>1,\*</sup> and Avinash Tiwari <sup>2</sup>

<sup>1</sup> Department of Botany, Government P.G. College Guna (M.P.) - 473001, India.

<sup>2</sup> School of studies in Botany, Jiwaji University, Gwalior, (M.P.) - 474011, India.

World Journal of Biology Pharmacy and Health Sciences, 2023, 16(02), 156–164

Publication history: Received on 27 September 2023; revised on 13 November 2023; accepted on 16 November 2023

Article DOI: <https://doi.org/10.30574/wjbphs.2023.16.2.0471>

### Abstract

This study aimed to investigate and contrast the amounts of carbohydrates and glycosides in several *Acacia catechu* bark extracts. Using samples collected over the course of two consecutive years, if any, the research also looked at how the seasons affected the same in the samples. This included a comparative analysis of six extracts using ethanol, methanol, aqueous solution, acetone, chloroform, and benzene to determine whether or not they contained glycosidic and/or carbohydrate components. The study's findings showed that soluble sugars and glycosidic chemicals were present in the sample regardless of the solvent system or the time of year it was collected. On the other hand, it was observed that all sample extracts were starch-free. The foundation for more research and the physiological effects of the same for the use of native plants for therapeutic reasons may be provided by this preliminary study.

**Keywords:** *Acacia catechu* bark extract; Carbohydrates content; Glycosides content; Qualitative analysis; Extracts; Seasonal dependent qualitative study

### 1. Introduction

As it is already well known, around 88% of the world's population uses plant-based remedies as their first line of defense while treating illness and maintaining health [1]. One of the biggest hubs and centers for the ancient knowledge of herbal medicine is India. Many Indian traditional herbal treatments have been improved and evaluated pharmacologically for decades before being included into the formal healthcare system [2]. Many secondary plant metabolites have commercial value and are components of many pharmaceutical products [3].

For many years, Ayurveda has extensively used *Acacia catechu*, a deciduous tree in the Fabaceae family, to cure and prevent a broad range of illnesses and/or ailments. In Hindi, it's known as Kahir, in English as Cutch tree, and in Sanskrit as Khadira [2, 3]. This plant's heartwood yields a very strong medicinal substance called Katha, which has several potential therapeutic uses. Saliva is coloured red when it contains *Acacia catechu* tree, which is used as a component in paan [4].

The several portions of *Acacia catechu* included tannins, phenolic compounds, alkaloids, saponins, flavones, sugars, and glycosides [4-6]. Additionally, it was observed to have very high concentrations of phenolic compounds, tannins, glycosides, and flavonoids, including procyanidin, quercetin, taxifolin, catechin/epicatechin, and epigallocatechin [5]. The most prevalent family of phenolic compounds found in nature are called flavonoids, and they may be found in a wide range of plant components in both free and glycoside forms, such flavonoid-3-o-glycosides [6].

\*Corresponding author: Archana Tiwari

From the ethyl acetate-soluble fraction of the ethanolic extract of *Acacia catechu* stems, a new bio-active flavone glycoside, C<sub>28</sub>H<sub>32</sub>O<sub>17</sub>, was isolated. Its structure was characterized as 5,7,3',4'-tetrahydroxy-3-methoxy flavone-7-O-β-D-galactopyranosyl-(1→4)-O-β-D-glucopyranoside. [7]. These potent substances, like epicatechin or catechin, have important anti-inflammatory and antioxidant properties. More precisely, this plant has a lot of catechin, which acts as an antibacterial and antioxidant. Particularly in Asia, the flavonoids included in *Acacia catechu* hardwood have been used for a variety of medicinal purposes, including antibacterial, anti-inflammatory, anti-viral, and cardiovascular [4, 6].

A tropical dry deciduous forest might be used to describe District Guna's Forest. Teak trees, such as *Butea monosperma*, *Diospyros melanoxylon*, *Acacia catechu*, *Acacia nilotica*, *Zizyphus numularia*, and *Terminalia bellerica*, are the predominant trees in these woods. As a result, *Acacia catechu*, which grows in the Guna region, was selected for this study's phytochemistry and possible therapeutic uses [8].

Previous studies have also shown the many positive health impacts of the various regional types of the plant sample under study; however, no data pertaining to the Guna district, which is a naturally occurring hub for the same, has been reported [9]. Nonetheless, a number of plant species, such as the bark of *Acacia catechu*, are often used as remedies in the Guna area despite the lack of supporting data from science [6, 8]. There is a lack of data, particularly concerning the screening of the carbohydrates and glycosidic component content of the same. Thus, it has been investigated whether the aforementioned characteristics are present in several solvent-based extracts of this plant's bark that were made using various techniques.

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## 2. Material and methods

### 2.1. Chemicals

α-naphthol, Benedict's reagent, Fehling's (A & B) solution, sodium nitroprusside, phosphoric acid, Toluene, Ethyl acetate, n-Hexane, Ethyl Ether, Ethanol, benzene, methanol, sodium bisulphate monohydrate, were supplied by Hi Media Laboratories Ltd., Mumbai, India. Ferrous sulphate, sodium chloride, sodium sulphate anhydrous, sodium hydrogen sulphate, sodium hydroxide, ferric chloride, hydrochloric acid, hydrogen peroxide, glacial acetic acid, and all other reagents were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd and from E-Merck (India) Ltd., Mumbai, India.

### 2.2. Collection and processing of bark samples

A random selection process was employed to gather the bark samples of *Acacia catechu* from trees located in the village of Biloniya, Guna, Madhya Pradesh. The collection area spanned a one-kilometer circle. In order to ensure uniformity, the selection of bark was consistently conducted from a vertical height of 1.3 meters above the surface [9]. A set of intact or uniformly shaped samples of bark were acquired; these were then cleaned manually before being weighed on a portable digital scale. A grand total of five plant samples were gathered during each of the three distinct seasons: winter (with a particular emphasis on mid-January), summer (exactly at mid-May), and autumn (specifically at mid-September). The data collection methodology spanned a period of two consecutive years, specifically 2016 and 2017. The materials that had been desiccated in a shaded area were pulverized in a controlled laboratory setting using a mechanical grinding apparatus set at room temperature. Subsequently, the particles were passed through filtration using a fine mesh with a pore diameter of 0.5 mm. Subsequently, the samples in pulverized form were preserved at a temperature of 4°C, under aseptic conditions [9, 10].

### 2.3. The preparation of bark extracts

The specimen under examination was partitioned into numerous aliquots in accordance with established protocols [10]. For the purpose of obtaining the aqueous extract, a pulverized bark sample weighing 50 grammes was extracted with 1000 milliliters of double-distilled water [11]. The extraction procedure was carried out at room temperature, utilizing a magnetic agitator to ensure uninterrupted agitation for a duration of three hours. Subsequently, the mixture was maintained in an undisturbed state for a period of twenty-four hours, as was previously executed. Following this, the mass of the filtrate was determined by subjecting it to a desiccation procedure and then weighing it. In a similar fashion, the organic solvents (80% ethanol, methanol, benzene, chloroform, and acetone) were prepared by combining a volume of 1000 milliliters with 50 grammes of desiccated fine powder obtained from the samples in a comprehensive manner at room temperature. In accordance with the method described above, every sample was extracted and subsequently dried [10, 12, 13]. Following this, the obtained desiccated extracts were preserved in a refrigerated environment at 4°C. In order to facilitate subsequent analysis and investigation, stock extracts were generated during the specified experimental phase by dissolving desiccated extracts in distilled deionized water (DDW) at a concentration of 1000 µg/ml [8, 11-13].

## 2.4. Qualitative analysis

The sample extracts were subjected to various qualitative chemical tests using standard protocols as mentioned below.

## 2.5. Test for carbohydrates

- **Molish test:** A fraction of a drop of alcoholic  $\alpha$ -naphthol solution was added to 2 ml of sample extract in a test tube. Then, 50 microliters of concentrated sulfuric acid were added along the test tube's walls. A violet colour ring formed at the interface of two liquids served as an indication that carbohydrates were present in the sample [6].
- **Benedict's test:** To conduct this test, 2 ml of sample extract was combined with 2 ml of Benedict's reagent in a test tube. The presence of carbohydrates was confirmed by the formation of a dark red, green or brown colour complex after the mixture was heated on a Bunsen burner for two to three minutes [6, 9].
- **Fehling's test:** For this experiment, 2 ml of sample extract was combined with 2 ml of Fehling's (A & B) solution in a test container. The resulting mixture was then heated on a Bunsen flame for five minutes. The presence of carbohydrates was indicated by the formation of a reddish-red precipitate [6,9].

## 2.6. Test for starch

**Iodine test:** Utilizing an iodine test, the presence of starch in the sample extract was ascertained. To accomplish this, a 2 mL sample extract was introduced into a test tube. The introduction of 100  $\mu$ l of iodine solution saturated with potassium iodine produced a dark blue coloration, which served as an indication of the existence of starch [9].

## 2.7. Tests for glycosides

- **Modified Bontrager's test:** One gramme of unrefined extract was weighed and transferred to a test tube for this procedure. To dissolve the extract, 5 ml of diluted HCl and 5 ml of 5% ferric chloride solution were added to this volume. The mixture was then chilled and filtered after being brought to a simmer in a water immersion for 10 to 15 minutes. Following benzene extraction of the filtrate, an equivalent volume of ammonia was introduced. Pink coloration indicated that anthraquinone glycosides were present in the sample [9].
- **Keller Killiani test:** In this experiment, 0.5 ml of ferric chloride solution was added to 2 ml of sample extract in a test tube containing 0.5 ml of glacial acetic acid. One milliliter of concentrated sulfuric acid solution was added along the test tube's walls after two minutes. A profound blue colour complex formed at the interface of the two liquids provided confirmation that cardiac glycosides (digitoxoside) were present [6, 9].

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## 3. Results and discussion

Considering that they are thought to be important in preventing sickness. To determine if different forms of sugars were present in the test sample as a whole, many qualitative tests were conducted. All samples were found to have carbohydrates in the test samples in all three tests, which are the Molish test, Benedict's test, and Fehling's test techniques. For each of these tests, all samples from all three seasons of both years showed almost identical colour intensity, or test parameter concentration (Table 1).

The Iodine test technique was used for the qualitative testing to determine the starch content. The results indicated that all extract samples tested had zero starch, including the bark samples from *Acacia* trees (Table 2). Studies have shown that the profiles of cell-wall polysaccharides in many fruits and vegetables change as they ripen, store, and undergo processing. This suggests that these polysaccharides play essential roles in the plants. For instance, *Cucurbita maxima* seeds have been shown in studies to be an excellent source of proteins, carbs, and other nutrients that promote health and to be able to treat benign prostatic hyperplasia [14].

However, the pumpkin seeds also showed significant levels of crude protein and carbs. Since it is well acknowledged that carbohydrates play a crucial role in plants as their primary energy sources, the carbon skeletons of organic molecules, and components of storage. The process of photosynthesis produces them [13, 15].

Furthermore, a critical role as hormone-like signalling molecules has developed and is being thoroughly studied at the moment. As they interact with diurnal fluctuations, abiotic and biotic challenges, and hormone signalling, they are also described as players in a complex communication system that is necessary for the coordination of metabolism with growth, development, and responses to environmental changes and pressures [12, 15, 16].

Previous studies in other plants have similarly confirmed the presence of carbohydrates in test extracts. There is growing evidence that carbohydrates function as antioxidants because they can scavenge reactive free radicals. Sugars might therefore be seen as necessary components of a well-functioning cellular redox network [6, 9, 10].

Current research has shown the medical applications of carbs as well as the fact that different types of carbohydrates are being investigated and evaluated for their capacity to boost resistance [17]. Among the biggest challenges to this method are the cuticle's low degree of penetration, which limits their perception, and the potential for alteration and/or metabolism by phyllo-sphere bacteria [1].

One insoluble form of polysaccharides is starch. The white, amorphous, odorless powder known as pure starch is insoluble in water and other typical organic solvents [9]. One of the most widely distributed chemical compounds in nature, it serves as the energy-storing form of plant components. Its insoluble nature in most solvents may be the cause of its inert behaviour, which is also evident in other studied unfavorable qualitative findings [12, 15].

Similar findings were seen in the qualitative testing for glycosides using the Keller Killiani test technique and the modified Bontrager's test method, as was previously noted [9]. Glycosidic substances were found in all of the samples [2]. For each of these tests, all samples from all three seasons of both years showed almost identical colour intensity, or test parameter concentration (Table 3). These findings align with what other studies have also seen.

**Table 1** Qualitative tests for carbohydrates using Molish test, Benedict’s test & Fehling’s test in different extracts of samples

Test for carbohydrates: Molish test (M), Benedict’s test (B) & Fehling’s test (F)																		
Sample types	Types of extracts																	
	Meth			Etha			Aque			Ace			Chlo			Bez		
	M	B	F	M	B	F	M	B	F	M	B	F	M	B	F	M	B	F
Sample 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Sample 21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sample 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Types of extracts - Math (methanolic extract), Etha (Ethanolic extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Sample 1-5 were collected in winter (January); 6-10 in summer (May) and 11-15 rainy season (September) in year 2016; Sample 16-20 were collected in winter, 21-25 in summer and 26-30 rainy season in year 2017.

**Table 2** Qualitative tests for starch using Iodine test method in different extracts of samples

<b>Test for starch: Iodine test</b>						
<b>Sample types</b>	<b>Types of extracts</b>					
	<b>Meth</b>	<b>Etha</b>	<b>Aque</b>	<b>Ace</b>	<b>Chlo</b>	<b>Bez</b>
Sample 1	-	-	-	-	-	-
Sample 2	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-
Sample 4	-	-	-	-	-	-
Sample 5	-	-	-	-	-	-
Sample 6	-	-	-	-	-	-
Sample 7	-	-	-	-	-	-
Sample 8	-	-	-	-	-	-
Sample 9	-	-	-	-	-	-
Sample 10	-	-	-	-	-	-
Sample 11	-	-	-	-	-	-
Sample 12	-	-	-	-	-	-
Sample 13	-	-	-	-	-	-
Sample 14	-	-	-	-	-	-
Sample 15	-	-	-	-	-	-
Sample 16	-	-	-	-	-	-
Sample 17	-	-	-	-	-	-
Sample 18	-	-	-	-	-	-
Sample 19	-	-	-	-	-	-
Sample 20	-	-	-	-	-	-

Sample 21	-	-	-	-	-	-
Sample 22	-	-	-	-	-	-
Sample 23	-	-	-	-	-	-
Sample 24	-	-	-	-	-	-
Sample 25	-	-	-	-	-	-
Sample 26	-	-	-	-	-	-
Sample 27	-	-	-	-	-	-
Sample 28	-	-	-	-	-	-
Sample 29	-	-	-	-	-	-
Sample 30	-	-	-	-	-	-

Types of extracts – Math (methanolic extract), Etha (Ethanol extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Sample 1-5 were collected in winter (January); 6-10 in summer (May) and 11-15 rainy season (September) in year 2016; Sample 16-20 were collected in winter, 21-25 in summer and 26-30 rainy season in year 2017.

**Table 3** Qualitative tests for glycosides using Bontrager’s test (modified) method & Keller Killiani test method in different extracts of samples

<b>Test for glycosides: Bontrager’s test (B) &amp; Keller Killiani test (K)</b>												
Sample types	Types of extracts											
	Meth		Etha		Aque		Ace		Chlo		Bez	
	B	K	B	K	B	K	B	K	B	K	B	K
Sample 1	+	+	+	+	+	+	+	+	+	+	+	+
Sample 2	+	+	+	+	+	+	+	+	+	+	+	+
Sample 3	+	+	+	+	+	+	+	+	+	+	+	+
Sample 4	+	+	+	+	+	+	+	+	+	+	+	+
Sample 5	+	+	+	+	+	+	+	+	+	+	+	+
Sample 6	+	+	+	+	+	+	+	+	+	+	+	+
Sample 7	+	+	+	+	+	+	+	+	+	+	+	+
Sample 8	+	+	+	+	+	+	+	+	+	+	+	+
Sample 9	+	+	+	+	+	+	+	+	+	+	+	+
Sample 10	+	+	+	+	+	+	+	+	+	+	+	+
Sample 11	+	+	+	+	+	+	+	+	+	+	+	+
Sample 12	+	+	+	+	+	+	+	+	+	+	+	+
Sample 13	+	+	+	+	+	+	+	+	+	+	+	+
Sample 14	+	+	+	+	+	+	+	+	+	+	+	+
Sample 15	+	+	+	+	+	+	+	+	+	+	+	+
Sample 16	+	+	+	+	+	+	+	+	+	+	+	+
Sample 17	+	+	+	+	+	+	+	+	+	+	+	+
Sample 18	+	+	+	+	+	+	+	+	+	+	+	+

Sample 19	+	+	+	+	+	+	+	+	+	+	+	+
Sample 20	+	+	+	+	+	+	+	+	+	+	+	+
Sample 21	+	+	+	+	+	+	+	+	+	+	+	+
Sample 22	+	+	+	+	+	+	+	+	+	+	+	+
Sample 23	+	+	+	+	+	+	+	+	+	+	+	+
Sample 24	+	+	+	+	+	+	+	+	+	+	+	+
Sample 25	+	+	+	+	+	+	+	+	+	+	+	+
Sample 26	+	+	+	+	+	+	+	+	+	+	+	+
Sample 27	+	+	+	+	+	+	+	+	+	+	+	+
Sample 28	+	+	+	+	+	+	+	+	+	+	+	+
Sample 29	+	+	+	+	+	+	+	+	+	+	+	+
Sample 30	+	+	+	+	+	+	+	+	+	+	+	+

Types of extracts – Math (methanolic extract), Etha (Ethanol extract), Aque (Aqueous extract), Ace (Acetone extract), Chlo (Extract in chloroform), Bez (Extract in benzene). Sample 1-5 were collected in winter (January); 6-10 in summer (May) and 11-15 rainy season (September) in year 2016; Sample 16-20 were collected in winter, 21-25 in summer and 26-30 rainy season in year 2017.

Plants generate a wide variety of secondary metabolites that may be "glycosylated," or embellished with sugars. Glycosylation occurs in plant metabolites for many reasons. For instance, glycosylated hydrophobic metabolites become more soluble in water, improving their metabolism and biodistribution [14]. The enhanced solubility and amphiphilicity of glycosylated metabolites may facilitate their easier passage across cell membranes. Furthermore, toxic compounds may be detoxified by glycosylation [7]. Glycosylation produces a non-toxic material that may be reactivated and used as an aglycone to defend against parasites and herbivorous animals. One example would be the cyanogenic glycosides that are produced by plants. These consist of a -hydroxynitrile group bonded to a D-glucose or other sugar moiety [17].

Plant-derived cardiac glycosides are secondary metabolites that have lactone rings functionalized at the 17- and 3-positions, respectively, on the steroid backbones. Cardenolides and bufadienolides are two subtypes of cardiac glycosides that are distinguished by the number of lactone rings in their rings—five or six [7, 9].

However, other research has identified related substances in other *Acacia* species, such as extracts from the bark of the *Acacia catechu* plant. It has been reported that cyanogenic glycosides and saponins are present in other *Acacia* plants, such as *Acacia sensu lato* and *Acacia arabica*. Saponins are derived from glycosidic molecules and have expectorant and cardiotoxic qualities [10, 11, 14]. According to some investigations, the bark of *Acacia catechu* and other *Acacia* species contains saponin chemicals. The hypoglycemic and anti-diabetic qualities of these saponins have been shown; these qualities are very beneficial in the management of diabetes mellitus [16, 18].

However, some research has shown that bean saponins may prevent cancer cells from growing by interfering with the cell's ability to replicate its DNA [7]. Cardiac glycosides are the drug of choice for treating congestive heart failure because of their well-established physiological impact. Furthermore, glycosides are known to have digestive, diuretic, and antibacterial effects [20].

Additionally, a study on glycosides found in other *Acacia* species, such as Acaciaside, a triterpenoid trisaccharide found in *Acacia auriculiformis*, and spinasterol, a phytosterol, was conducted. Another study also observed that myrtifoliosides A, B, and C were extracted as triterpene glycosides from the leaves of *Acacia myrtifolia* [21]. If there are any specific carbohydrates or glycosidic substances present in the sample extracts, further research will be necessary to determine whether or not they have any therapeutic effects.

#### 4. Conclusion

As a natural component, some of the carbohydrates and glycosidic compounds have known for their therapeutic activities. Since, this study provide primary data of the presence of above-mentioned compounds in test samples, further studies will be supported by this data. In addition, different extraction medium and season of sample collection were seen to untouched with the studied biomolecules, hence, the same also provide suitability of given extracts for upcoming

research work. Though the physiological consequences of the same remain unclear, more research is necessary to understand the amount, structure, and other properties of the same based on the data that is already accessible.

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## Compliance with ethical standards

### Acknowledgments

I express my sincere thanks to Dr. Sangeeta Sharma, Guest Faculty, School of Studies in Botany, Jiwaji University, Gwalior, (M.P.) who helped in data analysis.

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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