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Bacteriological Assessment of Some Traditional Herbal Formulations Sold in Sokoto Metropolis, Sokoto State Nigeria

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ABSTRACT

Traditional herbal formulations are widely used in Sokoto Metropolis for managing various ailments. However, concerns regarding their microbial safety are issues largely unaddressed. This study aimed at assessing the bacteriological composition of herbal formulations sold in Sokoto Metropolis, Sokoto State Nigeria. Powdered herbal samples were bought from various sales outlets and analysed under standard procedures. Total heterotrophic bacterial counts from the samples collected were enumerated. The morphological and biochemical characteristics of the isolates were analysed. Antibioqram test was then carried out on the pathogenic bacteria isolated. The results of bacterial counts ranges from 1.2×10^8 cfu/g to 5.0×10^6 cfu/g. A total of five (5) bacterial species were identified biochemically with varying frequency of occurrence as *Bacillus brevis* (10.5%), *Paenibacillus apiaries* (5.26%), *Staphylococcus aureus* (26.3%), *Bacillus cereus* (36.8%) and *Bacillus smithii* (21%). Five isolates of *S. aureus* were identified and each were given a code name as MTf, Fp5, MgP5, Psp5 and Olp5 respectively. The results of antibiogram test conducted on all the five isolates of *S. aureus* indicated that the isolates were susceptible to different antibiotics tested. Isolate FP5 showed the largest zone of inhibition for levofloxacin (41mm), indicating its high susceptibility, while other isolates exhibited varying resistance or susceptibility degrees each antibiotic. Amoxicillin (APX30 μ g) and Rifampicin (R25 μ g), showed no inhibition zone for all the isolates, indicating complete resistance. The presence of bacterial contaminants in herbal formulations poses a detrimental effect to human. Therefore, their pathological characteristics in herbal formulations underscores the need for stringent quality control measures to ensure consumers safety.

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INTRODUCTION

Herbal medicine also called botanical medicine or phytomedicine refers to the use of any plants, seed, berries, roots, barks, leaves or flowers for the treatment of illness (Braide *et al.*, 2013). Herbal medicines have gained its popularity among the people of different ages because of presumed low side-effect, low cost and a high level of acceptance by patients (Wilt *et al.*, 2000). Herbal medicine is of great importance to man and his health. More than 25% of the pharmaceutical drugs in use today originate from plant sources (Adeleye *et al.*, 2005). The World health organization (1998) has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. Herbal medicines in Nigeria are used in form of various preparations to treat various types of ailments including diarrhea, cough, neonatal fistula, convulsions, skin diseases, urinary tract infection, to decrease kidney stone; to lower cholesterol levels and blood pressure, also used as immune stimulants that help increase resistance to cold, relief from migraine headache and arthritis, healing of wounds; burns; skin ulcers; heart failure; hypertension, typhoid fever, malaria, infertility, fever, waist pain, chest pains, pile insomnia, ulcer, carbuncle, dizziness, blood prostration among others (Akunna *et al.*, 2023; Iyiola & Adegoke Wahab, 2024). Adeleye *et al.* (2005) emphasized that herbal drugs, apart from being unstandardized, they are cheap, accessible and also enjoy a wider acceptability among the people of developing countries.

Several Asian and African countries, including Nigeria have begun to encourage herbal medicine as an integral component of their public healthcare programmes (Ajagbonna & Onyeyili, 2002). Despite all the antecedent benefits attributed to plant materials and herbal medicine, a significant percentage of human and animal health problems can be traced to the consumption of plant materials either used as food or medicine. The injurious element in plant materials may come from the natural components of the plant or contaminants acquired during the process of preparation (Combest *et al.*, 2005). The World Health Organization survey indicated that about 70-80%

of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (WHO, 2009). In Nigeria, it was reported that not less than 70% of the population patronizes herbal medicinal preparations (Maiwada, 2004). Thus, large number of people in Nigeria rely on the use of traditional medicines for their health management, herbal products then must be of good quality, free of microbial contaminants and meets approved standard (Ekor, 2014). This study aims to carry out the microbial and phytochemical analysis of traditional herbal formulations sold in Sokoto metropolis.

The main concern regarding the use of traditional herbal medicine is lack of standardization (Izah *et al.* 2023). The fear over use of herbal drugs relates to the common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, most of them come with inadequate labelling and there is absence of appropriate patient information to elaborate on the above mentioned, many of the them remain untested and their use are either poorly monitored or not even monitored at all. Inadequate knowledge of their mode of action, potential adverse reactions, contraindications and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents.

Another major concern over quality of most finished products is mainly due to their potential contamination, considering their natural origin. (Combest *et al.*, 2005). Studies have reported contamination of herbal products with disease causing organisms such as *Bacillus* spp. *Salmonella* spp. and *Staphylococcus aureus* (Govender *et al.*, 2006). In Nigeria, though, there is proliferation of herbal products in the market, not so much has been done to guarantee the quality of the products produced for consumption. Some producers of herbal preparations in Nigeria do not have the required expertise to perform quality control on the preparation they produce. This brings about the problem of inconsistency on the quality of the herbal preparation in the country. With the ever-increasing use of herbal medicines and the

global expansion of the herbal medicines market, safety has become a concern for both health authorities and the public at large. This is because it is difficult to attest to the hygienic nature of the sanitary conditions from the point of collection of the herbs to processing and finally to the point of preparation.

The primary concern regarding the use of traditional medicine is safety, abiding by NAFDAC rules and regulations including route of administration and dose regimen. It has become necessary to create the conditions for correct and appropriate use of traditional medicines. There is the need to check if the populace in Sokoto are safe from the herbals they are consuming. This necessitated the research on microbial analysis of traditional herbal formulations sold in Sokoto metropolis. Therefore, this study aim to assess the bacteriological quality of some traditional herbal formulations sold in Sokoto metropolis, Sokoto state Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out within Sokoto metropolis, Sokoto State, Nigeria. Sokoto is the capital of Sokoto state, located in the extreme North Western part of Nigeria. According to the provisional figures of National Population Census, Sokoto has a population of about 5,307,154 (Males 2,706,649 and Females 2,600,506) suggestive of a population density of 97.7 persons per square Km. Majority of the population are Hausa/Fulani (Gada & Ismaila, 2021). The use of herbal medicines in Sokoto state has been documented for the management of diabetes (Shinkafi *et al.*, 2015), while for the treatment of malaria, peptic ulcer, and other ailments have been reported elsewhere (Oluranti *et al.*, 2012; Adebisi & Alebiosu 2014).

$$\text{Total colony counts (cfu/ml)} = \frac{nc}{vp} \times df$$

nc = No. of colony formed, vp = Volume plated, df = dilution factor.

Characterization and Identification of Bacterial Isolates

The isolates obtained were subjected to the following tests for their characterization and identification:

Inclusion Criteria

Packaged/processed powdered herbal samples were obtained from different shop outlets in Sokoto metropolis and were used to carry out the research.

Exclusion Criteria

Non-packaged/processed powdered herbal samples and liquid herbal samples were excluded from this study.

Collection of Samples

A total of one hundred and forty (140) finished/packaged/processed /powdered herbal formulations were obtained from several sale outlets in Sokoto metropolis and its environs. The samples includes: Olive Leaf Powder, Black seed powder, Fenugreek powder, Henna powder, Neem powder, Sesame powder, Moringa powder, Medicine for pile (*Binto koko*), Pepper mint powder (*Na'a Na'a*) and Typhoid herbs (*Maganin typhoid*). All samples were brought to the Microbiology laboratory of Usmanu Danfodiyo University, Sokoto for analysis.

Isolation and Enumeration of Bacteria from Herbal Samples

One gram (1g) each of the herbal samples were dissolved in 9 ml of sterile distilled water. This was thoroughly mixed and serially diluted up to 10^{-5} using 10-fold dilutions. Aliquots (0.1ml) of fifth-fold diluted herbal samples was inoculated onto sterile Petri dish containing prepared nutrient agar. The plates were incubated invertedly at 37°C for 24 hrs. Colonies emerged were sub-cultured on another nutrient agar to obtain pure culture of isolates which were characterized and identified based on their cultural, morphological and biochemical characteristics (Ismail *et al.*, 2018). Total colony counts were recorded in cfu/ml using equation:

Gram

This is a differential staining which classifies bacteria into gram positive or gram negative. Thin smears of fresh, pure bacteria cultures were made on a clean grease free glass slides. The slides were allowed to air dry and heat fixed by passing it over a Bunsen burner flame. The

slides were placed on a rack over a sink and flooded with crystal violet for 60 seconds, the slides were rinsed with clean tap water and flooded with iodine for 60 seconds, the slides were rinsed with water and briefly decolorized with 95% alcohol, the slides were counter stained with safranin for 60 seconds, rinsed and allowed to air dry after which they were viewed under oil immersion at (100x) objective lens (Oyeleke & Manga, 2008).

Catalase Test

One drop of three percent (3%) hydrogen peroxide was added on a glass slide. A loopfull of the test organism was placed on it using a sterile wire loop. A positive result was indicated by bubbling and frothing within ten (10) seconds (Cheesebrough, 2006).

Oxidase Test

An oxidase reagent (1% Tetramethylparaphenylene diamine dihydrochloride) was placed on the Whatman filter paper and a bacterial colony was smeared on the paper. The presence of the enzyme oxidase was observed by the appearance of a purple colour (Ochei & Kolhatkar, 2000).

Methyl Red and Voges-Proskauer Test

Five milliliter (5ml) of MR-VP broth was inoculated with the test organism and incubated for 24 hours at 37°C. After the period of incubation, one (1) ml of the broth was transferred to a small serological tube. Two (2) drops of methyl red was added and a red colour signifies a positive test while yellow signifies a negative test. Five (5) drops of 40% potassium hydroxide (KOH) followed by fifteen (15) drops of 5% naphthyl in ethanol was placed in a slanting position. The development of red colour starting from the liquid-air interface within an hour indicate a VP positive test. No change in colour indicate a VP negative test (Oyeleke & Manga, 2008).

Indole Test

The isolate was grown for 24 hours at 37°C in peptone medium and 0.5ml of Kovac's indole reagent was added into the culture and shaken. Presence of indole was confirmed by a formation of red ring colour on the surface of the medium after inoculating the organism (Cheesebrough, 2006).

Urease Test

A heavy inoculum from a 24-hour pure/slant culture was streaked on the entire slant surface. The caps of the tubes were loosened and incubated at 35°C for 24 hours and observed for a color change. Urease production is indicated by a bright pink (fuchsia) color on the slant which may extend into the butt (Brink, 2010).

Citrate Utilization Test

A speck of the isolates was streaked onto the surface of Simon's citrate agar and incubated at 37°C for 24 hours. The color of the slant was observed. Positive result was observed by the development of a deep blue colour (Oyeleke & Manga, 2008).

Sugar Fermentation Test

Colonies from a slant bottle was picked and sub cultured into nutrient agar medium contained in Petri dishes, followed by incubation at 37°C for 24 hours to obtain a fresh culture. Colonies of the fresh culture was picked and streaked on the surface of the slope of the triple sugar iron (TSI) media contained in test tube and then stabbed to the bottom using a straight wire loop. The inoculated media was then incubated at 37°C for 24 hours. Some microorganisms were able to ferment all the sugar turning the whole media yellow. Others were able to ferment one or more of the sugar indicated by change in colour of the media to yellow from red. Gas and hydrogen sulphide production was detected by the production of gas bubbles or cracking and blackening of the media respectively (Cheesebrough, 2006).

Motility Test

The isolate was inoculated onto Sulphide Indole Motility (SIM) medium and incubated at 37°C for 24 hours. Motility was observed by the spread of growth outwards from the stabbed area (Ochei & Kolhatkar, 2000).

Starch Hydrolysis Test

It is used to detect the ability of an organisms to hydrolyze starch by secreting the amylase enzyme in the surrounding media. The media was prepared and autoclaved at 121°C for 15 minutes and poured onto petri dishes and allowed to solidify. Using a sterile loop, a single line streak of the isolate was streaked across the plate and was incubated at 37°C for 24 hours. After the 24 hrs incubation, the plates were

flooded with gram's iodine and the plates observed were recorded (Gomes, 2020).

Antibacterial Susceptibility

The antibiotics used in this study were perfloxacin (10 µg), erythromycin (10 µg), amoxicillin (30 µg), levofloxacin (20 µg), CN, (10 µg), ampiclox (30 µg), Z (20 µg), R (25 µg), CPX (10 µg), AZ (12 µg). The standard Kirby-Bauer disk diffusion method was used to determine the susceptibility of the isolates. Antibiotic susceptibility was assessed using the disc diffusion method. Each pure culture was made into suspension and adjusted to 0.5 McFarland standards and inoculated aseptically on Mueller Hinton agar. Antibiotic discs were placed on Mueller Hinton Agar and incubated at

37°C for 24 hrs. Zones of inhibition were observed and measured with ruler after 24 hrs and compared with standard values (Obi *et al.*, 2024)

Statistical Analysis

All the data obtained were presented in frequency, percentage, mean, standard deviation (using in-built Microsoft excel) and tables.

RESULTS

Mean Bacterial Counts Herbal Drug Samples

Table 1 presents the result of the mean bacterial counts (CFU) of the sampled herbal powders. The highest mean CFU was observed in *Maganin Zazzabin Cizon Sauro* sample at 1.2×10^8 cfu/g while the lowest mean CFU was in black seed powder sample at 5.0×10^6 cfu/g.

Table 1: Mean Bacterial Counts Herbal Drug Samples

S/N	Sample	Colony Forming Units (CFU/g)
3	Zero Ulcer	$7.15 \times 10^7 \pm 10.60$
4	Oliver Leaf	$3.5 \times 10^6 \pm 0.70$
5	Magarya	$2.45 \times 10^7 \pm 6.36$
6	Moringa	$2.25 \times 10^7 \pm 2.12$
7	Black Seed	$5.0 \times 10^6 \pm 1.41$
8	Pure Senna	$9.0 \times 10^6 \pm 1.41$
9	Olive Leaf	$4.15 \times 10^7 \pm 3.53$
10	Fenugreek	$6.5 \times 10^6 \pm 2.12$
11	Cinnamon	$9.15 \times 10^7 \pm 0.70$
12	<i>Maganin typhoid fever</i>	$1.04 \times 10^8 \pm 5.65$
13	<i>Maganin Zazzabin Cizon Sauro</i>	$1.2 \times 10^8 \pm 23.33$

Keys; Mean±S.D

Morphological and Biochemical Characterisation of Bacterial Isolates

Table 2 highlights the morphological and biochemical identification of the bacteria isolated from the herbal samples. Five different

bacterial isolates were identified with varying occurrence frequency as *Bacillus brevis*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus smithii* and *Paenibacillus apiarius*.

Table 2: Morphological and Biochemical Characteristics of the Bacterial Isolates

S/No	Isolates	Gr Rxn	Cell Strc.	MR	VP	Indole	Urease	Catalase	Oxidase	Citrate	Gas	H ₂ S	Glucose	Lactose	Sucrose	Coagulas	Starch	Identified Bacteria
1	Mz5	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	<i>Bacillus brevis</i>
2	Mz5	+ve	Rod	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	<i>Paenibacillus apiarius</i>
3	Fp5	+ve	Cocci	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	<i>Staphylococcus aureus</i>
4	Zu5	+ve	Rod	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	<i>Bacillus cereus</i>
5	Zu5	+ve	Rod	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	<i>Bacillus smithii</i>

KEY: +ve = Positive Reaction -ve = Negative Reaction MR = Methyl Red VP = Voges Proskauer test H₂S = Hydrogen Sulphide Gr Rxn = Gram's

Frequency of Occurrence of the Bacterial Isolates

The frequency of occurrence of the bacterial isolates from the herbal formulations was presented in Table 3. *Bacillus cereus* was found to possess the highest frequency of 36.8%, followed by *Staphylococcus aureus* (26.3%), *Bacillus smithii* (21%), *Bacillus brevis* (10.5%), while *Paenibacillus apiaries* had the lowest occurrence of 5.26%.

Table 3: Frequency of Occurrence of Bacteria Isolated from Herbal Samples

S/N	Microorganisms	Frequency of occurrence	Percentage frequency of occurrence (%)
1	<i>Bacillus brevis</i>	2	10.5%
2	<i>Paenibacillus apiaries</i>	1	5.26%
3	<i>Staphylococcus aureus</i>	5	26.3%
4	<i>Bacillus cereus</i>	7	36.8%
5	<i>Bacillus smithii</i>	4	21%
	Total	19	100

Antibiogram Test on *Staphylococcus aureus* Obtained from Different Samples

Table 4 illustrates the results of antibiogram test conducted on different *Staphylococcus aureus* obtained. The zone of inhibition of the antibiotics measured in millimeters (mm), against the *Staphylococcus* isolates indicates the degree of susceptibility. Isolate FP5 shows the largest zone of inhibition for Levofloxacin

(41mm), indicating high susceptibility, while other isolates exhibit varying degrees of resistance or susceptibility depending on the antibiotic. Amoxicillin/clavulanic acid (APX30 μ g) and Rifampicin (R25 μ g), showed no inhibition zone for isolates, indicating complete resistance. Isolate PSP5, MGP2, and PLP5 were not inhibited by APX30 μ g.

Table 4: Antibiogram Test on *Staphylococcus aureus* Obtained from Different Samples

Zone of Inhibition (mm)

S/N	Codes	A	B	C	D	E	F	G	H	I	J
1	PSP	14	28	16	4	-	15	-	13	26	11
2	FP	31	41	42	18	-	12	-	12	33	17
3	PLP	13	21	25	11	-	11	-	-	11	-
4	MTF	12	21	27	11	-	14	-	11	25	17
5	MgP	-	16	8	-	-	8	11	21	18	3

Keys: A= E10 μ g, B= LEV20 μ g, C= Pef10 μ g, D = CN10 μ g, E = APX30 μ g, F = Z20 μ g, G = AM30 μ g, H=R25 μ g, I=CPX10 μ g, J=Az12 μ g.

Discussion

Without proper regulation and quality control, consumers remain vulnerable to contaminated herbal mixtures because the presence of harmful bacterial species in these products have been known to lead to serious infections, food poisoning, and long-term health complications.

Therefore, this research seek to dive into the bacteriological assessment of some of these herbal formulations. The result of bacterial colony counts of different herbal formulations sold in Sokoto metropolis was presented in Table 4.1 where the counts of Black seed powder herbal formulation was recorded as

$5.0 \times 10^6 \pm 1.41$, which was found to be the least prevalent among the samples obtained. As noted by Afam-Ezeaku *et al.* (2025) and Chiegeiro *et al.* (2022), the presence of high load of bacterial contamination might be due to failure of applying sterilization concepts in herbal drug formulation or the materials used in preparing the herbal medicines. This finding is closely similar to finding of Abba *et al.*, (2009) in a research titled "Contamination of herbal medicinal products marketed in Kaduna metropolis with selected pathogenic bacteria" whose average colony count result was recorded as 5×10^7 cfu/g. The limits of bacterial contamination given in European pharmacopoeia as reported by Okunlola *et al.* (2007) are: total aerobic bacteria (10^5 cfu/g), Enterobacteria and other Gram negative organisms (10^3 cfu/g) and *Escherichia coli* and *Salmonella* should be absent. The herbal products under study did not meet these specifications in most cases because the bacterial counts ranged between 1.2×10^8 cfu/g to 5.0×10^6 cfu/g.. Other possible sources of contaminants are the personnel(s) that could introduce the bacteria when handling the raw materials during processing. Therefore, the process of harvesting, drying, storage, handling and the soil influence the bacteriological quality of raw material which in turns affects the entire quality of the herbal preparation.

The bacterial isolates identified from the morphological and biochemical tests were depicted in Table 2. The biochemical reactions of the isolates indicate a variety of metabolic capabilities. For example, isolates like Mz5(2) and Mz5(1) exhibited positive reactions for catalase, urease, and citrate utilization, but differ in the ability to ferment glucose or lactose. In contrast, isolates Tf5 and Zu5 (1) were MR and VP positive. The varied biochemical profiles suggest that the isolates represent different bacterial genera or species. The presence of catalase and urease activities in isolates could point to organisms like *Staphylococcus* or *Proteus*, while the ability to ferment glucose and produce hydrogen sulfide is often associated with *Enterobacteriaceae* or *Salmonella* species. Gas, H₂S production, and sugar fermentation profiles add another layer of specificity to the

identification process. Isolate Fp5(2) and Zu5(1), which produce gas and H₂S, might belong to anaerobic or facultatively anaerobic species that can ferment carbohydrates and produce sulfur compounds as metabolic byproducts. On the other hand, isolates such as Mp5(1) and Mp5(2), which show negative results in multiple tests, could be characterized as more biochemically inert or less metabolically versatile. In this study, the bacteria identified were *Bacillus brevis*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus smithii* and *Paenibacillus apiarius*. This work is similar to the work of Larsson & Flach (2022). This study revealed that *Bacillus* spp. were the predominant bacteria in the herbal medicines. They assessed Nepal herbal medicines and reported that all herbal medicines tested were contaminated with *Bacillus* spp. which are commonly found in the soil, air, dust etc. Many aerobic species of *Bacillus* produce endospore that helps them not only to resist environmental stress but also to ensure their long term survival under adverse conditions.

The ubiquitous nature of bacteria was also observed in the herbal samples obtained through their frequency of occurrence (Table 3). From this study, *Bacillus cereus* was found to possess the highest frequency of 36.8%, highlighting that majority of the bacteria obtained were *Bacillus cereus*. *Staphylococcus aureus* was found to be the second most dominant with frequency of 26.3%, followed by *Bacillus smithii* (21%), *Bacillus brevis* (10.5%), while *Paenibacillus apiaries* had the lowest occurrence of 5.26%. As summarized in this research, *Bacillus* species were the predominant genera isolated, which is in line with the finding of the sensitivity test on *Staphylococcus aureus* is similar to the work of Abba *et al.* (2009). Antibiotic susceptibility studies on the bacterial contaminants of the powdered herbal products indicated that the isolates were susceptible to the antibiotics tested. This was not unexpected as it has been previously observed that organisms such as Methicillin Resistant *Staphylococcus aureus* (MRSA) which are known to be resistant to multiple antibiotics, tend to be susceptible to multiple antibiotics when they are of environmental origins (Shukla *et al.*, 2004; Wylie and Nowicki, 2005). The near uniform

antibiotic susceptibilities of the isolates could therefore be because they are of environmental origins and agrees with the observation of Akindahunsi *et al.* (2021) that environmental bacterial isolates are susceptible to most antibiotics because resistance transfer is thought to be much less efficient in the environment where microorganisms are widely separated by distance as compared with human and animal intestines.

CONCLUSION

The result of this research illustrated bacterial presence across the different herbal powders, underscoring the need for careful evaluation of these substances for medicinal use. *Maganin Zazzabin Cizon Sauro* was found to possess the highest count of 1.2×10^8 cfu/g, while local black seed possess the lowest count (5.0×10^6 cfu/g). Five bacterial species were identified with varying frequency of occurrence where *Bacillus cereus* had the highest frequency of occurrence. From the antibiogram test conducted on different *Staphylococcus aureus* identified, the species showed varying susceptibility against different antibiotics. Therefore, these results underscores the need for continuous surveillance of *Staphylococcus aureus* resistance patterns to guide appropriate antimicrobial therapy and minimize the spread of resistant strains.

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