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# ARTICLE Preservative Effect of *Newbouldia laevis* (Boundary Tree) Leaf Extract on Shelf-Life of Fresh Chicken Meat under Tropical Conditions

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ARTICLE INFO	ABSTRACT						
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Keywords: Aqueous extracts Antimicrobial Broiler chicken Newbouldia laevis Meat quality Heat stress	<i>N. laevis</i> on preservation of fresh chicken meat under tropical condition is scanty and thus, investigated. Broiler chicken meat (10kg weight) was obtained immediately after slaughter and were randomly allotted to three treatments (T1- control; T2- aqueous extract of wet leaves of <i>N.</i> <i>laevis</i> ; and T3- aqueous extract of dry leaves of <i>N. laevis</i> ) in a Random- ized Complete Block Design. Tropical plant, such as <i>Newbouldia laevis</i> with relatively high resistance to heat stress, possesses viable bioactive compounds that can lower the growth of spoilage micro-organisms and activities of reactive oxygen species on fresh chicken meat under trop- ical conditions for 48 hours. Hence, poultry farmers in developing na- tions with fluctuating power supply can adopt the quick meat shelf life enhancement technique, while commercial poultry farmers across the globe can embark on product fortification using extracts of <i>Newbouldia laevis</i> .						

# 1. Introduction

Relatively high temperatures have favoured spoilage micro-organisms in catalyzing the process of meat quality deterioration <sup>[1]</sup>. Transit time needed by consumers from product purchase stations to their homes is another limitation of meat quality, as meat quality control during this period is practically impossible and may involve fluctuations in temperature and relative humidity. Chicken meat gets spoiled quickly in the presence of an atmosphere comprised of air. This is caused by the rapid growth of pseudomonades, resulting in lowered shelf life of meat. The influence of oxidative deterioration and microbial activities are showcased in the reduction of shelf life, physico-chemical and organoleptic properties of fresh carcass. Researches have shown that lipid oxidation and microbial growth in meat products can be controlled by using either synthetic or natural food additives <sup>[2,3]</sup>. Plants possessing antioxidant and antimicrobial properties

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have the advantage of being readily accepted by consumers, as they are considered natural and safe. Hence, the use of cheap, but highly effective anti-oxidants and antimicrobial substances become imperative in ensuring sustainable animal product processing.<sup>[4]</sup> noted that the presence of spoilage microorganisms in meat can accelerate lipid oxidation and produce changes in the organoleptic properties of meat. According to <sup>[5]</sup>, meat spoilage bacteria use soluble compounds such as glucose and amino acids contained in muscle tissue for growth, with high preference for glucose. Meat surface layers with considerable amount of glucose will prevent further degradation by bacteria, but if absent, an attack on amino acids is observed with release of unpleasant odour from generated organic suphides and amines.<sup>[6]</sup> reported that meat spoilage is most frequently caused by Pseudomonas spp., Enterobacteriaceae, Brochothrix thermosphacta, and Lactic acid bacteria.

Psychrotrophic species such as *Pseudomonas fragi*, *P. lundensis*, *P. putida* and *P. fluorescens* can be isolated from unpacked meat showing signs of spoilage. The *P. fluorescens* occurs more frequently on fresh meat, though during longer periods of storage, *P. fragi* becomes dominant. However, tropical plant such as *Newbouldia laevis* posess antibacterial and antioxidative compounds that could limit microbial spoilage in meat samples.

Newbouldia laevis (Bignoniaceae) is a sun-loving, fast-growing, drought-tolerant species from west tropical Africa. It is used traditionally for diarrhoea, dysentery, dropsy, swellings, oedema, and gout; and as febrifuges or genital stimulants/depressants. Also, the plant is held in high esteem by Yoruba and Hausa cabinet, thus preventing the indiscriminate destruction of such specie of weeds. Pharmacological studies on extracts of different parts of N. *laevis* have revealed the antioxidant <sup>[7]</sup> and antimicrobial <sup>[8]</sup> activities among others. <sup>[7]</sup> reported that the phytochemical constituents of different parts of N. laevis revealed the presence of alkaloids, phenylpropaniod glycosides, flavonoids, tanins, saponins, phenols, essential oils, terpenoids, triterpenoids, quinoids, and ceramides among others. Micro organisms' resistance to drugs has been prevalent and as such, has necessitated the adoption of natural alternatives such as the exploration of phytochemicals in meat preservation. Oxygen reactive species have been found to participate in a growing number of diorders by causing oxidative damage to meat and meat products, thereby necessitating the use of compound with anti-oxidant activity. The need for meat product fortification becomes imperative, as different disorders have emanated from contaminated food products. Therefore, this research was conducted to determine the proximate composition of fresh broiler chicken meat treated with wet and dry leaf extracts of *Newbouldia laevis*; and to investigate the anti-oxidative and anti-microbial effects of wet and dry leaf extract (aqueous) of *Newbouldia laevis* on the shelf life of fresh broiler chicken meat.

# 2. Materials and Methods

The study was carried out at the Animal Products and Processing Laboratory, after the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee, through the Department of Animal Science, University of Ibadan, Nigeria. Broiler chicken meat with average weight of 10kg was obtained immediately after slaughter and washed with water to remove dirt, before placing on shelf. Chicken samples (breast meat) were cut and randomly allotted to three treatments (T<sub>1</sub>- control; T<sub>2</sub>- aqueous extract of wet leaves of N. laevis; and  $T_3$ - aqueous extract of dry leaves of N. laevis) in a Randomized Complete Block Design. The leaves of N. laevis was phyto-chemically screened for bioactive chemical substances. Wet leaves (500g) of N. *laevis* were harvested, slightly washed and mechanically pressed into 5 litres of distilled water, and another portion was air-dried for 7 days, for wet and dry aqueous extract solution preparation, respectively. Meat immersion technique was adopted in the treatment of fresh chicken samples as described by the authors. At 0, 12, 24, 36 and 48 hours, data on microbial load (x  $10^{-4}$  CFU) were determined by the agar well diffusion method. The analysis was run using the following Agars: Nutrient agar for aerobic bacteria, Mackonky agar for coliform determination, and Potato Dextrose Agar (PDA) for fungi. The agars were measured and dissolved in distilled water according to the instructions of the manufacturers. A 1g meat sample from each treatment was weighed and mashed in 9ml of distilled water to give a uniform mixture. Serial dilution method was used in which 10ml of  $10^3$  and  $10^5$  of each sample was pipette onto a sterile petri dish and the already prepared agar at 45 °C was poured into it. It was swirled gently for even distribution; the plates were inverted and incubated in an incubator at 38°C for 24 hours after which the colonies formed on each media was counted using the visual aid. Oxidative damage were obtained using standard procedures. Proximate composition of meat samples was done using the procedure of A.O.A.C.<sup>[9]</sup>. Lipid oxidation using Thiobarbituric acid relative substance (TBARS), water holding capacity (WHC) and colour of meat samples were done using standard procedures. Data were analysed using descriptive statistics and analysis of variance in the SAS<sup>[10]</sup> software package at a probability level of 0.05

### 3. Results

# 3.1 Proximate Composition and Phytochemical Screening

The proximate composition (as wet basis) of broiler chicken meat on extracts of leaves of Newbouldia laevis is shown in Table 1. Crude protein (CP) content in broiler chicken meat on extract of Newbouldia laevis was significantly (P<0.05) affected at 12 hours of treatment. Higher (P<0.05) CP was observed in meat on control (26.71) compared to wet (22.99) and dry (23.57) leaf extract. Ash content was significantly (P<0.05) altered by extract of Newbouldia laevis and ranged from 0.35 (dry) to 0.77 (control). Ether extract observed in broiler chicken meat sample on wet extract (3.83) of Newbouldia laevis was significantly (P<0.05) lower compared to dry (4.34) extract. The highest (P<0.05) ether extract was observed in chicken meat on control (4.98). Crude fibre observed in chicken sample on wet leaf extract (0.13) was significantly (P<0.05) higher compared to dry leaf extract (0.10), but both were significantly (P<0.05) lower compared to the control (0.24). Broiler chicken meat samples on aqueous extract of wet (70.95) and dry (71.11) leaf extract of Newbouldia laevis were significantly (P < 0.05) higher in moisture content compared to those on control (63.21%). Qualitative evaluation of phytochemicals present in leaves of Newbouldia laevis is shown in Table 2. Secondary metabolites such as saponin, tannins, terpenoids, alkanoids, flavonoids, and phenols were present in dry and wet leaf of Newbouldia laevis.

 Table 1. Proximate composition (as wet basis) of broiler

 chicken meat on Newbouldia laevis leaf extract treatments

		Newbould		
Parameters (%)	Control	Wet leaf extract	Dry leaf extract	SEM
Crude protein	26.71 <sup>a</sup>	22.99°	23.57 <sup>b</sup>	0.06
Ash	0.77 <sup>a</sup>	0.46 <sup>b</sup>	0.35°	0.01
Ether extract	4.98 <sup>a</sup>	3.83°	4.34 <sup>b</sup>	0.01
Crude fibre	0.24 <sup>a</sup>	0.13 <sup>b</sup>	0.10 <sup>c</sup>	0.002
Moisture content	63.21 <sup>b</sup>	70.95 <sup>a</sup>	71.11 <sup>a</sup>	0.06

*Note:* <sup>abc</sup> Means of treatments along a row with different superscript differed significantly (P<0.05) using DMRT. SEM-standard error of means. Number of replication: 5

 
 Table 2. Qualitative phytochemical constituents of Newbouldia laevis leaf extracts

Phytochemicals	Wet leaf	Dry leaf		
Saponin	+	+		
Tannins	+	+		
Terpenoides	+	+		
Alkanoids	+	+		
Flavonoids	+	+		
Phenolic	+	+		

Note: + means present.

### **3.2 Microbial Population in Fresh Broiler Chicken Meat**

Table 3 shows the effect of Newbouldia laevis leaf extracts on microbial population of chicken meat. At slaughter, total bacterial count (TBC,  $x \ 10^{-4}$  CFU ) in fresh chicken meat under different treatments did not differ (P>0.05) significantly, and ranged from 1.50 (wet extract) to 2.60 (control). At 12 hours, significantly (P<0.05) higher TBC was observed in control meat sample (15.50) compared to wet (6.40) and dry (4.65) leaf extracts of Newbouldia laevis. At 24 hours, no significant (P>0.05) differences were observed in TBC of chicken samples subjected to wet (7.70) and dry (6.89) leaf extracts of Newbouldia laevis. However, higher (P<0.05) TBC was observed in the control meat sample (24.85). At 36 hours, higher (P<0.05) TBC was observed in the control meat sample (39.30), and was significantly (P<0.05) higher compared to meat samples on wet (9.60) and dry (8.80) leaf extracts of Newbouldia laevis. However, at 48 hours, significantly (P<0.05) lower TBC was observed in chicken meat samples on dry (17.70) leaf extract of Newbouldia laevis compared to wet (28.90) and the control (64.00).

Total fungi count (TFC) observed in chicken meat after slaughter were not significantly affected (P>0.05) by different treatments. However, at 12 hours, lower (P<0.05) TFC was observed in meat samples on wet (1.82) and dry (1.34) leaf extract treatments compared to the control (5.20). Similar observations were recorded at 24, 36 and 48 hours, with increasing (P<0.05) TFC recorded in the control meat samples (22.80) compared to wet (6.20) and dry (5.40) leaf extracts of *Newbouldia laevis*.

Newbouldia laevis							
Spoilage organ- isms	Shelf life (hours)	Control (T1)	Wet extract (T2)	Dry ex- tract (T3)	SEM	P value	
TBC (x 10 <sup>-4</sup> CFU)	0	2.60	1.50	1.75	0.18	0.52	
	12	15.50 <sup>a</sup>	6.40 <sup>b</sup>	4.65 <sup>b</sup>	1.45	< 0.0001	
	24	24.85 <sup>a</sup>	7.70 <sup>b</sup>	6.85 <sup>b</sup>	1.08	< 0.0001	
	36	39.30 <sup>a</sup>	9.60 <sup>b</sup>	8.80 <sup>b</sup>	1.32	< 0.0001	
	48	64.00 <sup>a</sup>	28.90 <sup>b</sup>	17.70 <sup>c</sup>	3.68	< 0.0001	
TFC (x 10 <sup>4</sup> CFU)	0	1.14	0.29	0.32	0.05	0.46	
	12	5.20 <sup>a</sup>	1.82 <sup>b</sup>	1.34 <sup>b</sup>	0.37	< 0.0001	
	24	8.00 <sup>a</sup>	3.64 <sup>b</sup>	2.56 <sup>b</sup>	0.09	0.002	
	36	9.90 <sup>a</sup>	4.78 <sup>b</sup>	4.10 <sup>b</sup>	0.06	0.0001	
	48	22.80 <sup>a</sup>	6.20 <sup>b</sup>	5.40 <sup>b</sup>	1.66	< 0.0001	

**Table 3.** Effect of *Newbouldia laevis* leaf extract on microbial population of fresh broiler chicken meat

*Note:* <sup>abc</sup> Means of treatments along a row with different superscript differed significantly (P<0.05) using DMRT. TBC- Total bacteria count, TFC- Total fungi count, SEM- Standard error of means, P value- probability.

# **3.3 Oxidative Properties and pH of Fresh Broiler** Chicken Meat Samples

Table 4 shows the effect of Newbouldia laevis leaf extract on the pH and oxidation properties of broiler chicken meat. It was observed that the pH of meat samples at slaughter was significantly (P<0.05) affected by different treatments. Higher (P<0.05) pH was observed in the control (6.13) compared to meat on dry (5.84) leaf extract but did not differ (P>0.05) significantly from wet (5.97) extract. At 12 hours, pH recorded for meat on control (6.30) was significantly (P<0.05) higher compared to wet (6.07) and dry (6.03) leaf extract. Similar pH records were observed in chicken carcass at 24 hours. However, at 36 hours, meat samples on dry leaves extract (6.37) had significantly (P<0.05) lower pH compared to other treatment. Oxidation in broiler chicken meat samples was not significantly (P>0.05) influenced by different leaf extracts of Newbouldia laevis at slaughter and 12 hours on shelf, and ranged from 0.14 (wet and dry leaf extract) to 0.34 (control). However, at 24 hours, significantly (P<0.05) higher thiobarbituric acid relative substance (TBARS) value was observed in chicken meat samples on control (0.62) compared to wet (0.32) and dry (0.35) leaf extracts. Higher (P<0.05) TBARS value was observed in meat samples on control at 36 hours and 48 hours (7.98) compared to wet (0.65) and dry (0.63) leaf extracts.

 Table 4. The pH and oxidative properties of fresh broiler chicken meat as affected by Newbouldia laevis leaf extracts

Newbouldia laevis							
Parame- ters	Shelf life (hours)	Control	Wet extract	Dry extract	SEM	P value	
	0	6.13 <sup>a</sup>	5.97 <sup>ab</sup>	5.84 <sup>b</sup>	0.07	0.02	
	12	6.30 <sup>a</sup>	6.07 <sup>b</sup>	6.03 <sup>b</sup>	0.04	0.003	
рН	24	6.90 <sup>a</sup>	6.42 <sup>b</sup>	6.19 <sup>b</sup>	0.15	0.001	
	36	7.67 <sup>a</sup>	6.61 <sup>b</sup>	6.47 <sup>c</sup>	0.05	< 0.0001	
	48	7.98 <sup>a</sup>	6.99 <sup>b</sup>	6.58°	0.13	< 0.0001	
TBARS	0	6.13 <sup>a</sup>	0.14	0.14	0.04	0.07	
	12	6.30 <sup>a</sup>	0.29	0.26	0.08	0.12	
	24	6.90 <sup>a</sup>	0.32 <sup>b</sup>	0.35 <sup>b</sup>	0.11	0.002	
	36	7.67 <sup>a</sup>	0.48 <sup>b</sup>	0.47 <sup>b</sup>	0.09	0.004	
	48	7.98 <sup>a</sup>	0.65 <sup>b</sup>	0.63 <sup>b</sup>	0.15	0.002	

*Note:* <sup>abc</sup> Means of treatments along a row with different superscript differed significantly (P<0.05) using DMRT. SEM- standard error of means, P value- probability. TBARS- Thiobarbituric acid relative substance.

The water holding capacity (WHC) of broiler chicken meat, as affected by aqueous extract of *Newbouldia laevis* is shown in Table 5. It was observed that chicken meat samples on wet (73.75) and dry (74.13) leaf extracts of *Newbouldia laevis* were significantly (P<0.05) higher in WHC compared to the control (53.54) at slaughter. Similar observations were recorded at shelf life intervals em-

ployed. At 48 hours, the WHC observed in chicken meat samples on the control (58.80) was significantly (P<0.05) lower compared to wet (67.90) and dry (66.80) leaf extracts.

Lightness of meat on control sample (51.19) was significantly (P<0.05) higher compared to wet (43.87) and dry (42.48) leaf extracts of *Newbouldia laevis*. Redness of meat was significantly (P<0.05) lower in meat samples on wet (10.18) and dry (11.30) leaf extract compared to the control (21.05). However, yellowness of chicken meat was not significantly (P>0.05) affected by aqueous extract of *Newbouldia laevis*.

 Table 5. Effect of Newbouldia laevis leaf extract on water

 holding capacity and colour of broiler chicken meat

Newbouldia laevis							
Parameters	Shelf life (hours)	Con- trol	Wet extract	Dry ex- tract	SEM	P value	
	0	53.54 <sup>b</sup>	73.75 <sup>a</sup>	74.13ª	1.56	< 0.0001	
	12	60.80 <sup>c</sup>	83.10 <sup>a</sup>	73.00 <sup>b</sup>	2.17	< 0.0001	
WHC (%)	24	56.00 <sup>b</sup>	77.00 <sup>a</sup>	73.20 <sup>a</sup>	3.03	< 0.0001	
	36	52.80 <sup>b</sup>	73.00 <sup>a</sup>	72.20 <sup>a</sup>	1.06	< 0.0001	
	48	58.80 <sup>b</sup>	67.90 <sup>a</sup>	66.80 <sup>a</sup>	2.45	< 0.0001	
Colour (HU)							
Lightness		51.19ª	43.87 <sup>b</sup>	42.48 <sup>b</sup>	1.41	0.004	
Redness		21.05 <sup>a</sup>	10.18 <sup>b</sup>	11.30 <sup>b</sup>	1.61	0.002	
Yellowness		31.16	36.35	33.68	2.23	0.30	

*Note:* <sup>abc</sup> Means of treatments along a row with different superscript differed significantly (P<0.05) using DMRT. WHC- Water holding capacity, SEM- standard error of means, P value- probability.

#### 4. Discussion

Some plants contain valuable antioxidants and antibacterial compounds of great nutritional and therapeutic values against various food-borne microorganisms <sup>[1]</sup>. From current study, crude protein (CP) content in chicken meat on extract of Newbouldia laevis was significantly affected at 12 hours of treatment with higher CP observed in meat on control. These results have shown that an increase in crude protein value observed in the control could have been as a result of microbial activities, as meat protein tends to increase with higher degrading ability of spoilage microbes. Reduction in meat ether extract in present study might have contributed to the decrease in lipid peroxidation, coupled with the anti-oxidative effect of leaves of Newbouldia laevis. These findings were in agreement with the report of Mittler [11] who observed that reactive oxygen species (ROS) participate in a variety of chemical reactions with biomolecules leading to oxidative stress. The author stated further that under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into oxygen through mitochondrial respiration. The ROS percentage increases during infections, exercise, stress, exposure to pollutants, UV light, among others, leading to increase in oxidative stress in tissues <sup>[12]</sup>. Similar observations were reported by Niki *et al.* <sup>[13]</sup> who noted that ROS reactions with biomolecules produce different types of secondary radicals depending on the nature of the ROS. These radicals in the presence of oxygen are converted to peroxyl radicals, which often induce chain reactions. Atmani et al. [14] reported that flavonoids play important role in protecting biological systems, and against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA. Reports have revealed that plants are rich sources of natural antioxidants <sup>[15]</sup>. The result of present research confirms the report of Gill and Molin<sup>[16]</sup> who observed that aerobic spoilage flora growth is slowed down by high concentrations of carbon dioxide. However, the genera that dominate the spoilage flora of meat stored in air will still predominate in aerobic atmospheres modified by the addition of carbon dioxide. The authors stated further that the only means of precluding that dominance is to exclude oxygen from the atmosphere around the meat. In the current study, meat immersion technique postulated have proven effective in reducing temperature as well as reducing atmospheric oxygen around the meat samples, thus limiting the proliferation of these spoilage flora. Silva et al. <sup>[17]</sup> reported that phenolic acids possess antimicrobial activity against some strains of bacteria and function as antioxidant. The presence of these secondary metabolites in leaves of N. laevis was responsible for the reduced growth of spoilage micro-organism associated with meat spoilage.

From current study, similar effect of aqueous extract of Newbouldia laevis was observed alongside reports of authors on the antimicrobial potential of methanol extract of the leaf <sup>[8]</sup>. The results obtained from present study could also be due to the presence of essential oils, as plant extracts obtained from aromatic medicinal plants have been reported to show antimicrobial effects against bacteria, filamentous fungi, yeasts, and viruses. From current study, extracts of leaves of Newbouldia laevis significantly lowered pH of meat, thereby improving its shelf life. Pungent odour, a characteristic property of spoilage in meat was not observed in meat treated in aqueous extract of Newbouldia laevis. This result agrees with the report of Harborne and Tomas-Barberan<sup>[18]</sup> who stated that many of the terpenoid containing substances are commercially interesting because of their use as flavours and fragrances in foods and cosmetics, as they are important for the quality of agricultural products.

From present findings, chicken meat samples on wet

and dry leaf extract of Newbouldia laevis were significantly higher in WHC compared to the control. The ability of meat to hold its water or added water is crucial to the handling and sensory properties of meat. This result have shown that meat samples treated with aqueous extract of Newbouldia laevis were improved and would give reduced or no excessive exudate in packaging and loss of weight during cooking and on processing. From the study conducted, lightness of meat on control sample was significantly higher compared to wet and dry leaf extract of Newbouldia laevis. Redness of meat was lower in meat samples on wet and dry leaf extract of Newbouldia laevis. The reason for the observed differences could have been as a result of the influence of chlorophyll contained in leaves of plants. Nevertheless, visual examination showed pleasant preference for meat samples on dry leaf extract.

# **5.** Conclusion

At 12 to 48 hours, aqueous extracts of wet and dry leaves of *Newbouldia laevis* significantly reduced microbial population in chicken meat under tropical conditions. Also, oxidative rancidity in chicken meat samples was lowered significantly with *Newbouldia laevis* wet and dry leaf extracts. However, meat treated in aqueous extract of *Newbouldia laevis* were lower in texture and higher in cooking loss. Aqueous extract of dry leaves of *Newbouldia laevis* enhanced chicken meat colour intensity and efficiently increased the shelf life of fresh broiler chicken meat at 48 hours post-slaughter.

#### Novelty and Importance of the Research

The shelf life of chicken meat has been rapidly reduced as a result of high environmental temperatures prevalent in the tropics which favored the activities of spoilage micro-organisms, and reactive oxygen species that function in oxidative damage. In developing countries with low power supply, it becomes imperative to maximize farmers' profit, ensure meat safety standards while adopting a quick but cheap remedy to incidences of heat stress and meat spoilage in poultry industry. Leaves of *Newbouldia laevis* contained phytochemicals that prolong the shelf life of fresh chicken meat under tropical conditions.

Hence, poultry farmers in developing nations with fluctuating power supply can adopt the quick meat shelf life enhancement technique, while commercial poultry farmers across the globe can embark on product fortification using extracts of *Newbouldia laevis*.

#### **Conflicts of Interest**

The authors declare no conflicts of interest as regarding

the publication of this paper

# **Informed Consent**

The authors confirm that written consent was obtained from all participants prior to the study.

#### **Ethical Approval**

The study received the ethical approval of the Institutional Animal Care and Use Committee, through the Animal Products and Processing Unit of the Department of Animal Sciences, University of Ibadan, Nigeria.

#### Contributorship

O. R. Popoola designed and implemented the research. I. O. Popoola analyzed data and drafted the manuscript. O. Olusola supervised the research. All authors approved the final draft of the manuscript.

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