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ANTIBACTERIAL EFFECT OF FIVE SELECTED NIGERIAN MEDICINAL PLANT EXTRACTS

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ABSTRACT The search for new drugs to combat infectious diseases and emergence of resistant microorganisms stimulated this research. This study was therefore designed to screen and compare five selected medicinal plants extracts used in Nigeria as traditional medicine for their antibacterial effects. These plants include *Harungana madagascariensis* Lam. Ex Poir and *Enantia chlorantha* Oliv. barks, *Senna alata* Linn., *Gossypium hirsutum* Linn. and *Alstonia bonnie* De Wild leaves. Agar well diffusion method was carried out to test Ethanol extracts against reference strains of *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Tube dilution method was carried out to determine both Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts. Data were analysed using ANOVA at p = 0.05. *Harungana madagascariensis*, *E. chlorantha* and *S. alata* crude extracts exhibited broad spectrum antibacterial activity by inhibiting all the tested bacterial species with 24.3±0.3, 25.7±0.3 and 27.7±0.6 (mm) in diameter zones of inhibition respectively. The MIC of the extracts ranged from 5.0 to 20.0 mg/mL while the MBC ranged from 20.0 to 30.0 mg/mL. *Enantia chlorantha*, exhibited MIC of 5.0mg/ml against *S, aureus* and *P. aeruginosa*. *Enantia chlorantha* and *A. bonnie* showed MBC of 20mg/ml against *P. aeruginosa* and *E. coli* respectively.
The five selected plant extracts exhibited antibacterial activities which justify their use for treatment in traditional medicine. They could serve as alternative sources of antibacterial agents against resistant strains of microorganisms.

Keywords: Medicinal plants, Extracts, Antibacterial, Inhibition, Broad spectrum, Microorganisms

INTRODUCTION

Natural products are the fascinating varieties among the innumerable gifts of nature and have being inseparable parts of human history. Various plant species contain complex and essential organic components which have been vastly utilized for therapeutic purposes. The utilization of therapeutic agents in Nigeria which constitute completely plants matter is an evidence of a long history of interaction between human and the environment. Medicinal plants are simply plant matter utilized by humans as therapeutic agents to treat and inhibit the occurrence of diseases (Heinrich and Jagar, 2015). The therapeutic potency of these plants is as a result of intrinsic bioactive components that elicit specific physiological activity on the health of humans (Shintu *et al.*, 2015). Rural communities of most developing countries depend greatly on utilization of these medicinal plant species for the treatment of wide range of diseases and these plant species have indeed exhibit wide range of antimicrobial potency (Abdelhalim *et al.*, 2017). Most of these medicinal plant species utilized across different nations of the world are closely related or similar (Salinitro *et al.*, 2017).In developing countries like Nigeria, bacterial infections are still the main cause of deaths (Ljungham *et al.*, 2016; White *et al.*, 2019 and Onajobi *et al.*, 2020). The emergence of the inability of chemotherapeutic agents and the resistance of pathogens to antibiotics have made the screening of plant species an alternative to the treatment and prevention of diseases. (Fentahum *et al.*, 2017). *Enantia chlorantha* Oliv, often called the African yellow wood, is a tropical rainforest tree, which possesses a fluted stem, thin and brown enternal part of bark with pale cream coloured inner bark. It is a type of plant species that is effective against diseases such as tuberculosis malaria and so (Tcheghebe *et al.*, 2016).

Harungana madagascariensis Lam., also known as dragon's blood tree is a shrub commonly found in Africa having ovate lateral leaves. This shrub has a maximum height of 1.65m (Kouam *et al.*, 2006) and it is often referred to as *"Aroje"* by the Yoruba speaking populace in southwest region of Nigeria while the igbos called it "*Uturu"*. It is often found in regions where annual rainfall that is more than 1300mm. Both the bark of its stem and also the leaves has been locally utilized as therapeutic agents for the treatment of malaria, anaemia gastrointestinal diseases and so on (Iwalewa *et al.*, 2008).

Alstonia boonei De Wild is a widely distributed species of medicinal plant that is found in most part of the world specifically in rain-forest areas, belonging to the *Apocynaceaee* family. It has been vastly used for the treatment of varieties of ailments which include

insomnia, chronic diarrhea, rheumatic pains playing various antagonistic role such as antipyretic, anti-venom and anti-inflammatory activities (Adotey *et al.*, 2011, Orwa *et al.*, 2009).

Gossypium hirsutum, Linn. (*Malvaceae*) is a cotton plant species cultivated across the world, often referred to as "Owu" in southwest region of Nigeria (Ezuruike and Prieto, 2014). According to Center for New Crops and Plants Products (Center fir New Crops and Plants Products, 2002), *G. hirsutum* can grow in areas such as along road sides, areas of river overflow, well inland and drained soils and also grows optimally with a pH range of 5.2 to 7.0. The epidermal and cortex tissues of cotton roots secrete extracellular high level of gossypol (Scheffler, 2016). Its therapeutic properties are highly recognized in Asia, Africa and North America (Triplett *et al.*, 2008).

Senna alata Linn (Fabaceae) belong to the shrub species which is often referred to as "Candle stick". It is a perennial shrub, commonly found in West Africa that optimally grows in tropical rain forest. In the Nigerian Herbal Pharmacopoiea it is a legalize antimicrobial component (NHP, 2008), which is usually utilized for the treatment of wide range of diseases in Nigeria which include skin infections, lung diseases, allergic diseases, and so on (Chatterjee *et al.*, 2012). The leaves also play roles in the treatment of gonococci diseases, oedema, abdominal pains and so on (Sule *et al.*, 2011).

Although many antibiotics are available for the treatment of infectious diseases, their uses are limited due to low potency, poor solubility, emergence of resistance strains and drug toxicity (Wang *et al.*, 2017). In recent years, drug producing companies have focused on producing antimicrobial components from biological matter. Medicinal plants have continued to be the most effective and cost less source of antibiotics (Atanasov *et al.*, 2015). Drug discovery must be a continuing process if effective chemotherapeutic agents against the rapidly increasing drug resistant bacteria and fungi are to be obtained.

The current study aimed at evaluating and compares the antibacterial activities of five selected indigenous medicinal plantstowards the treatment of infectious pathogens.

MATERIALS AND METHODS

Plant Collection, Identification and Authentification

Barks of *Harungana madagascariensis* Lam. Ex Poir and *Enantia chlorantha* Oliv and leaves of *Senna alata* Linn., *Gossypium hirsutum* Linn. And *Alstonia boonei* De Wild were obtained from a farmland in Idi-Ayunre, Ibadan, Oyo State in March, 2010. The plants were selected based on literature review and their traditional uses in the treatment of infectious diseases. Plantswere identified at Herbarium, Department of Botany, University of Ibadan, Ibadan, Nigeria, and authenticated at Forest Research Institute of Nigeria (FRIN) with specimen vouchers issued. Identification numbers of FRI-109553, FRI-109554, FRI-109555, FRI-109556 and FRI-109557, were assigned to *Enantia chlorantha*, *Harungana madagascariensis*, *Alstonia boonei*, *Gossypium hirsutum* and *Senna alata* respectively.

Plant Processing

Freshly collected plant materials were air dried under the shade to avoid heat and moisture by spreading and constantly re-spreading to allow air circulation. This process was continued until the plant materials were sufficiently dried. The resulting materials changed colour from green to brown. An electric blender with Model number MS-223, was used to powder the dried plant materials into powder. The resulting powder was then stored in a plastic container for future use.

Extraction of Plant Materials

Cold extraction method was carried out with ethanol. The pulverized plant materials (5.0 kg) poured into big conical flask (5 L) and were submerged with distilled 5 L ethanol for the period of 72 hours. These were filtered with Watmann No. 1 Filter paper and were further soaked repeatedly until it turned colourless. The filtrate was evaporated under reduced pressure at 25ºC. The resulting 500 g crude extracts of each sample were collected and later stored in a refrigerator.

Antibacterial Activity

The test organisms used which included *Staphylococcus aureus (*ATCC25923)**,** *Escherichia coli (*ATCC25922), *Pseudomonas aeruginosa* (POA 286), *Salmonella typhi* (ATCC 14028), *Bacillus subtilis* (ATCC 6633) and *Shigella flexneri* (ATCC 12022) were collected from H.E.J. Research Institute, International Centre for Chemical and Biological Sciences, Karachi, Pakistan.

Standardization of Inoculum

The turbidity of the inoculum was adjusted through the use of McFarland standard. Standard preparation was achieved by mixing 0.5 mL of 1.75% barium chloride di-hydrate solution with 95.5 mL of 1% sulfuric acid. An optical density between 0.08 and 0.1 was used to determine the McFarland standard precise density which was measured spectrometrically at 625 nm. An average count of X-colony forming units per mL was enhanced by the adjusted turbidity of the suspension. At 37ºC, bacterial test culture was incubated overnight. The resulting growth was then compared with the 0.5 McFarland standards.

Antibacterial Susceptibility Test

The susceptibility of test organisms to plant extract was examined using the modified agar well diffusion method, described by (Ajala *et al.*, 2016). Twenty eight grams of Nutrient agar (Oxoid) was dissolved in 1L distilled water. Homogenized using microwave and later autoclaved at 121ºC for 15 minutes. After cooling to about 45ºC, 50 mL of the medium was poured in sterile 14 cm diameter Petri plate. This was then allowed for proper solidification of the medium and kept at room temperature for 24 hours to check the sterility of the prepared medium. Nutrient Broth and Soft Agar were equally prepared in the same manner with 0.8 gm dissolved in 100 mL of distilled water with approximately 2 mL and 7 mL respectively was dispense in screw capped test tubes before autoclaved at 121ºC for 15min and later kept at room temperature.

The bacteria species under study were inoculated on Muller-Hinton II agar (Oxoid) and subjected to incubation at 35ºC. For further analysis, slants were also prepared which is then stored at 4ºC. A colony of the resulting growth was sub-cultured for 18 hours into a nutrient broth. In order to obtain 10^6cfu per mL of bacterial culture, dilution up to 10^5 folds was carried out. 100 ml of the culture was introduced into a sterile soft agar tube and this was mixed thoroughly. Aseptically, the mixed culture was inoculated into growth media which constitute solidified nutrient agar to form lawn. In order to ensure thorough distribution of test organism, the plate was gently swirled. With the aid of a sterile cork borer wells were created in the solidified medium. The wells were labeled. 1mg/ml of the test compound was dissolved in DMSO with 100 µL of test compound in each well plate, according to bacterial culture in triplicates. The wells were separately filled with antibiotics and DMSO which act as positive and negative controls. For proper diffusion, the plates were kept for 30 min. this is then incubated at 37ºC for 24 hrs. A transparent ruler was used to measurein millimeter the zones of inhibition. The average was obtained as the mean of the triplicate (Ajala *et al.*, 2016).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was represented by least concentration of plant extracts that inhibited the growth of test microorganisms. The determination of MIC of the plant extracts at different concentrations in mg/mL was carried out using broth dilution method (Ajala *et al.*, 2016). Briefly 5, 10, 15 and 25 mg of plant extracts was dissolved in 1 mL DMSO to form appropriate stock solutions in mg/mL. From each concentration, 0.1 mL of plant extract stock solutions was prepared and transferred to test tubes which contain nutrient broth containing 9 mL of the test organisms and subjected to incubation 37ºC for 24 hours. Control was set up whereby test organisms was immersed in sterile distilled water instead of plant extracts and the plant extracts was without test organisms. The tube containing the lowest concentration of the plant extracts which shows no apparent growth after incubation for 2 days was taken as MIC.

Minimum Bactericidal Concentration (MBC)

According to the procedure carried out by (Mostafa *et al.*, 2017), the minimum bactericidal concentration of the plant extracts on the test organisms was obtained. 1ml of the suspension obtained during the analysis of MIC, was transferred to a solidified nutrient agar and incubated for 24hrs. The lowest extract concentrations which show no apparent growth was taken as the minimum bactericidal concentration.

Data are mean of three replicates ± SEM and were subjected to Duncan's Multiple Range test using Statistical Package for the Social Sciences, (SPSS 15.0).

RESULTS

Table 1 shows the diameter of zones of inhibition of bacterial growth at varying concentrations of *H. madagascariensis, E. chlorantha, G. hirsutum, A. boonei* and *S. alata* extracts measured in millimeter (mm). For *S. aureus* (ATCC 25923), zones of inhibition increased significantly ($p < 0.05$) from 20.00 ±0.57 to 26.66 ±0.33 and from 14.00 ±0.33 to 19.66 ±0.33 with increasing concentrations from 5 mg/mL to 25 mg/mL of *S. alata* and *G. hirsutum* extracts respectively. Zones of inhibition appeared constant with increasing concentrations of *H. madagascariensis* and *E. chlorantha* extracts from 5 mg/mL to 25 mg/mL. The extracts of *H. madagascariensis, E. chlorantha* and *S. alata* were effective against *S. aureus* ATCC 25923 at lower concentration of 5 mg/mLwith zones of inhibition 21.33 ±0.57, 21.33 ±0.57 and 20.33 ±0.57 respectively.

Senna alata, *A. boonei* and *E. chlorantha* were most effective at 5 mg/mL against *E. coli* ATCC 25922, *P. aeruginosa* POA 286 and *S. flexineri* ATCC12022with zones of inhibition 20.33 ±0.57, 25.66 ±0.33 and20.33 ±0.33respectively. The zone of inhibition significantly increased from 20.33 ±0.33 to 29.00 ±0.57as the concentration of *E. chlorantha* extracts increased from 5 mg/mLto 25 mg/mL against *S. flexineri* ATCC12022. The *E. chlorantha* extracts exhibited significant zone of inhibition, 20.33 ±0.57 against *S. typhi* ATCC 14028at concentration of 15 mg/mL. The zones of inhibition 12.33 ±0.33, 16.33 ±0.57 and 20.33 ±0.57were significant against *S. typhi* ATCC 14028for

S. alata, *H. madagascariensis* and *E. chlorantha* extracts at 5, 10 and 15 mg/mL respectively. The zone of inhibition 29.00 ±1.00 exhibited by *A. boonei* extracts against *P. aeruginosa* POA 286 was greater than 23.00 mm zone of inhibition exhibited by imipinem (standard drug).

Table 1: Zones of Bacterial Growth Inhibition at Different Concentrations of Selected Plant Extracts

Conc. (mg/mL)	Extracts	*S. aureus*	*E. coli*	*P. aeruginosa*	*Sh. flexineri*	*Sal. typhi*
5	HMB	21.00 ±0.57a	8.00 ±0.00c	18.33 ±0.33c	7.66 ±0.33c	7.66 ±0.33b
	ECL	21.00 ±0.57a	10.33 ±0.33b	24.33 ±0.33b	20.33 ±0.33a	8.66 ±0.33b
	SAL	20.33 ±0.57a	20.33 ±0.57a	40.33 ±0.33a	13.66 ±0.33b	12.33 ±0.33a
	GHR	14.33 ±0.33b	7.33 ±0.33cd	9.00 ±0.00d	13.66 ±0.33b	8.66 ±0.33c
	ABN	8.33 ±0.33c	6.66 ±0.33d	23.66 ±0.33c	8.66 ±0.33c	0.00 ±0.57c
10	HMB	21.00 ±0.57a	10.33 ±0.00c	20.33 ±0.57b	13.66 ±0.33b	15.33 ±0.57a
	ECL	23.66 ±0.57a	13.66 ±0.33b	29.00 ±0.57b	23.66 ±0.33a	10.00 ±0.33b
	SAL	23.66 ±0.33a	15.33 ±0.33a	42.66 ±0.57a	15.66 ±0.33b	13.66 ±0.33a
	GHR	15.33 ±0.33b	10.66 ±0.33c	15.66 ±0.33d	15.66 ±0.33b	10.00 ±0.33c
	ABN	6.33 ±0.33c	7.66 ±0.00d	25.66 ±0.33c	10.00 ±0.00c	0.00 ±0.33c
15	HMB	19.66 ±0.33c	10.33 ±0.33d	21.00 ±0.00c	14.33 ±0.33d	17.00 ±0.33a
	ECL	22.00 ±0.57a	14.33 ±0.33b	29.66 ±0.33b	25.66 ±0.33a	20.33 ±0.33a
	SAL	25.66 ±0.33a	21.66 ±0.33a	45.33 ±0.33a	17.66 ±0.33c	15.66 ±0.33c
	GHR	15.66 ±0.33d	12.66 ±0.33c	16.66 ±0.33c	16.66 ±0.33b	12.00 ±0.33b
	ABN	7.33 ±0.33e	7.33 ±0.33e	27.66 ±0.33b	14.66 ±0.33d	12.66 ±0.33d
25	HMB	20.33 ±0.33b	11.66 ±0.33d	21.00 ±0.33d	15.66 ±0.33d	15.66 ±0.33b
	ECL	20.33 ±0.33b	15.66 ±0.33c	29.66 ±0.33a	27.66 ±0.33a	20.00 ±0.57a
	SAL	26.66 ±0.33a	23.66 ±0.33a	46.00 ±0.00a	17.66 ±0.33c	17.66 ±0.33a
	GHR	19.66 ±0.33b	15.66 ±0.33c	21.66 ±0.33c	16.66 ±0.33d	13.66 ±0.33c
	ABN	9.66 ±0.33c	21.33±0.33b	24.66 ±0.33b	13.66 ±0.33e	0.00 ±0.33d
Imipenem	0.010	38.00	25.00	23.00	18.00	00.00

Key: HMB- *Harungana madagascariensis*, ECL- *Enantia chlorantha*, SAL- *Senna alata*, GHR- *Gossypium hirsutum*, ABN- *Alstonia boonei*, - = not determined, Values were mean of three determinations ± S.E.M. Values in each vertical column carrying different letters are significantly different from one another ($p < 0.05$) for each concentration.

expressed lowest bacteristatic and bacteriocidal concentrations at 5 mg/mL and 20 mg/mL respectively with lowest MBC/MIC ratio of 4.0 against *P. aeruginosa* (POA 286).

The extracts of *H. madagascariensis* and *Senna alata* had bacteristatic concentrations at 8 mg/mL and 10 mg/mL and bacteriocidal concentrations at 22 mg/mL and 25 mg/mL respectively, while their MBC/MIC ratios were 2.75 and 2.50 respectively against *P. aeruginosa* (POA 286). The bacteristatic activity expressed by *E. chlorantha* was significant against *S. flexineri* ATCC 12022 and *S. typhi* ATCC 14028 at concentration of 10 mg/mL each than other plant extracts. The MBC/MIC ratios exhibited by *E. chlorantha* against *S. flexineri* ATCC 12022 and *S. typhi* ATCC 14028 were 2.5 and 2.50 respectively. *G. hirsutum* and *S. alata* extracts expressed their bacteristatic effect at concentration of 15 mg/mL each while *H. madagascariensis* and *A. boonei* extracts expressed their bacteristatic effect at 20 mg/mL concentration each against *S. flexineri* ATCC 12022. It was observed that *H. madagascariensis*, *E. chlorantha* and *S. alata* extracts expressed their bacteristatic effect against *S. typhi* ATCC 14028at 10, 10 and 15 mg/mLrespectively.

Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentrations (MBC) of selected Plant Extracts

In table 2, *E. chlorantha*, *H. madagascariensis* and *Senna alata* exhibited minimal bacteristatic concentrations at 5 mg/mL, 6 mg/mL and 6 mg/mL and minimal bacteriocidal concentrations at 22 mg/mL, 24 mg/mL and 20 mg/mL against *S. aureus*(ATCC 25923) respectively. The MBC: MIC ratios exhibited by *E. chlorantha*, *H. madagascariensis* and *Senna alata* were 4.4, 4.0 and 3.3 respectively against *S. aureus* ATCC 25923. For *E. coli* ATCC 25922, the minimal bacteristatic concentrations at 8 mg/mL, 9 mg/mL and 10 mg/mL and minimal bacteriocidal concentrations at 25 mg/mL, 20 mg/mL and 25 mg/mL exhibited by *E. chlorantha*, *Senna alata* and *H. madagascariensis* respectively, while their MBC/MIC ratioswere expressed as 3.1, 2.2 and 2.5 respectively against same *E. coli*. *Alstonia boonei* and *E. chlorantha* extracts

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of selected Plant Extracts in mg/mL

		In vitro Antibacterial Activity				
		Test Isolates				
Fractions	Conc.	*S. aureus*	*E. coli*	*P. aeruginosa*	*Sh. flexineri*	*Sal. typhi*
HMB	MIC	6.0	10	8.0	20	10
	MBC	24	25	22	30	25
	MBC/ MIC ratio	4.0	2.5	2.8	1.5	2.5
ECL	MIC	5.0	8.0	5.0	10	10
	MBC	22	25	20	25	25
	MBC/ MIC ratio	4.4	3.1	4.0	2.5	2.5
SAL	MIC	6.0	9.0	10	15	15
	MBC	20	20	25	20	30
	MBC/ MIC ratio	3.3	2.2	2.5	1.3	2.0
GHR	MIC	10	20	20	15	N
	MBC	25	30	30	25	N
	MBC/ MIC ratio	2.5	1.5	1.5	1.6	-
ABN	MIC	15	20	5.0	20	20
	MBC	>30	>30	20	>30	30
	MBC/ MIC ratio	>2	>1.5	4.0	>1.5	1.5

Key: HMB- *Harungana madagascariensis*, ECL- *Enantia chlorantha*, SAL- *Senna alata*, GHR- *Gossypium hirsutum*, ABN- *Alstonia bonnie* , N- not determined *S. a- Staphylococcus aureus; E. coli- Escherichia coli; P. a- Pseudomonas aeruginosa; Sh. f – Shigella flexineri; Sal t- Salmonella typhi;* - no inhibition

DISCUSSION

The results obtained showed that lower concentrations of plant extracts tested were effective against *S. aureus* (ATCC 25923) and *P. aeruginosa* (POA 286) at 5 mg/mL. The result showed significant ($p < 0.05$) increase in the zones of inhibition when treated with the extracts of *S. alata* and *G. hirsutum* while the zones of inhibition appeared constant with increased concentrations of extracts from *H. madagascariensis* and *E. chlorantha*. *P. aeruginosa* POA 286, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhi* ATCC 14028but not to *S. flexineri* (ATCC 12022) were susceptible to extracts of *H. madagascariensis.* The susceptibility of Gram positive *S. aureus* to the extracts from *H. madagascariensis* correlates with the findings of (Iwalewa *et al.,* 2009) but different in activity reported for the extracts against gram negative *P. aeruginosa*.

Alstonia boonei and *E. chlorantha* extracts showed significance activity against *P. aeruginosa* (POA 286) when compared to other tested plant extracts. The potency expressed by *A. boonei* extracts against Gram negative *P. aeruginosa* (POA 286), probably due to chemical constituents which include alkaloids, triterpenoids and steroids (Adotey *et al.,* 2012). The antibacterial potency exhibited by the extracts of *E. chlorantha* against *S. typhi* (ATCC 14028)in this study was highly significant compared to the result of (Adesokan *et al.*, 2007), who reported 100 mg/mL and 150 mg/mL as the bacteriostatic and bacteriocidal concentrations respectively as against 10 mg/mL and

25 mg/mL concentrations reported in this study. Aqueous extract of dried stem bark of *E. chloranthai* is effective against the antagonistic activity of viruses and it has been used to treat diseases such as treat jaundice and urinary tract infections (Maurya and Singh, 2014, Tcheghebe *et al.,* 2016).

CONCLUSION

In this study, the selected plant extracts have been shown to be bacteristatic in nature. The plant extracts exhibit the potency of broad spectrum antibiotics which are active against both gram-positive and gram negative bacteria that were under study. This potency is as a result of the phytochemicals compounds they produce. This, therefore prove their useful as antimicrobial components and thus, plant can be continuously utilized for the treatment of most bacterial infections and diseases. Further research work needs to be carried out on these plant extracts to reveal their bioactive constituents and to study the mode of actions of these plant extracts. This will enhance quick discovery of new and active chemotherapeutic drugs for the treatment of infectious diseases.

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