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In Vitro Antibacterial Activity of *Vernonia amygdalina* and *Croton macrostachyus* Leaf Extracts against Selected Human Pathogenic Bacteria

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Abstract

Humans have used plants to cure various disorders throughout their history. *Vernonia amygdalina* and *Croton macrostachyus* are among the commonly used medicinal plants in various parts of Ethiopia. This study evaluated the antibacterial potential of two plants against some standard bacteria. Plants materials were collected from natural vegetation, air-dried under shade, ground into powder, and soaked in separate Erlenmeyer flasks. The extract mixture was shaken by rota-evaporator for 3 days. The extracts were screened for phytochemicals. Methanolic leaf extracts of *V. amygdalina* revealed bacterial zone of inhibition (19.5 ± 1.5 mm) at 200 mg/mL against *Staphylococcus aureus*. Similarly, *C. macrostachyus* revealed inhibition (18.0 ± 0.0 mm) at 200 mg/mL on *S. aureus*. On the contrary, the lowest inhibition zone was obtained from the extract of *C. macrostachyus* on *S.typhi* at 25 mg/mL. Metanolic extract of *C. macrostachyus* revealed significantly higher inhibitions against *S. aureus* than *K. pneumoniae* , *S. typhi* and *E. coli* ($p < 0.05$). Extract of *V. amygdalina* showed the lowest minimum inhibitory and bactericidal concentration at 6.25 and 12.5 mg/mL against *S. aureus* respectively. The findings indicate that extracts of *V. amygdalina* and *C. macrostachyus* possess potential antibacterial activities against selected human pathogenic bacteria.

Keywords: *Croton macrostachyus*; Minimum inhibitory concentration; Phytochemical screening; *Vernonia amygdalina;* Inhibition zone.

1. Introduction

Pathogenic bacteria are major causes of diseases in the world, particularly in developing countries [1]. Food and water bone bacteria are among the common bacteria which cause human diseases [2].

Natural products, particularly derived from plants, played essential roles in the prevention and treatment of diseases throughout history. Evidence suggests that nearly 80% of people in developing countries depends on traditional plants derivatives to meet healthcare needs [3].

In Ethiopia, limited access to modern medical services, shortages of medicines might be the cause for the use of traditional remedies [4]. However, many medicinal plants used in traditional medicine have not been scientifically evaluated for their safety, efficacy, and mechanisms of action.

Among the medicinal plants commonly used in Ethiopia are *V. amygdalina* in Amharic ("Grawa") and *C. macrostachyus* ("Bisana"). *V. amygdalina* was traditionally used for the treatment of infections and other ailments [5]. *C. macrostachyus* also widely used in traditional medicine across Africa, including Ethiopia [6]. Despite their traditional use, there is limited scientific evidence regarding their antibacterial activities against human pathogenic bacteria. Thus, the study aimed to evaluate the phytochemical constituents and antibacterial activities of *V. amygdalina* and *C. macrostachyus* leaf extracts against selected human pathogenic bacteria.

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2. Methods

2.1 Study design

Laboratory experimental -based study was employed from October 2021 to May 2022 at the Microbiology Laboratory in Debre Markos University, Ethiopia.

2.2 Plant materials and test microorganisms

The leaves of *V. amygdalina* ("Grawa") and *Cr*oton *macrostachyus* ("Bisana") were used in this study. Fresh leaves were collected from Debre Markos town, northwestern Ethiopia. The samples were washed, air-dried under shade for two weeks, pulverized into a fine powder, passed through a 0.50 mm mesh sieve, and stored in sterile glass containers for further use.

Standard bacterial reference strains were obtained from the Ethiopian Public Health Institute. The bacterial cultures preserved under at 4°C until they were used for the experiments.

2.3 Preparation of plant extracts

Two hundred grams of plant powder were separately macerated in 500 mL of distilled water and 99.9% methanol and shacked for1wo days. The extracts were filtered and concentrated using a rota-evaporator. The crude extracts were stored at room temperature for further analysis [7].

2.4 Preparation of extract concentrations

Crude samples were serially diluted to obtain concentrations of 200, 100, 50, and 25 mg/mL. About 10 µL extracts were loaded to 6 mm disc yielding extract doses of 2.0, 1.0, 0.5, and 0.25 mg per disc, respectively [8].

2.5 Antibacterial activity

Agar disc diffusion methods were employed to evaluate bacteria activities. Fresh bacterial cultures were adjusted with 0.5 MacFarland to obtain approximately 1×10^7 CFU/mL. Subsequently, 1 µL suspension was swabbed onto Mueller–Hinton agar (MHA) plates containing the test extracts, following the method described by [9-10]. The bacterial samples were swapped onto Mueller–Hinton agar, and extract-loaded discs were placed on the agar using Ciprofloxacin (30 µg) and distilled water as positive and negative controls, respectively. The triplicated plates were placed at 37°C for 24 hours, and the zones of inhibition were measured in millimeters [11].

2.6 MIC determination

MIC of the extracts was employed using serial dilutions ranging from 100 to 6.25 mg/mL. After inoculation, the cultures were incubated at 37°C for 24 hours. MIC was determined with the lowest concentration inhibiting bacterial growth [12].

2.7 MBC determination

MBC was determined by sub-culturing samples from MIC according to procedure carried out by [13].

2.8 Phytochemical screening

Qualitative phytochemical analyses of the extracts were conducted to determine the presence of saponins, flavonoids, alkaloids, tannins, phenols, and terpenes using standard procedures described by [14-16].

2.9 Data analysis

The antimicrobial susceptibility was computed by using one way ANOVA in the contrast select the polynomial, in post hoc select LSD and tukey and in the options part by selecting description the data was analyzed ($p \leq 0.05$ significant values).

3. Results

3.1. Extracts Yield

The highest yield (18.6 %) was obtained from methanol extract of *V. amygdalia* , while the lowest from aqueous extract of *C. macrostachyus* which was 10.4% (Table 1).

Table 1 Crude extract yields *V. amygdalia* and *C. macrostachyus*

Plant type / plant part	Solvent	Yield (g)	Yield (%)
C. macrostachyus / leaves	MeOH	8.0	16.0
C. macrostachyus / leaves	H_2O	5.2	10.4
V. amygdalina / leaves	MeOH	9.3	18.6
V. amygdalina / leaves	H_2O	6.1	12.2

Footnote:H2O=Aqueous and MeOH= Methanol

3.2. Evaluation of Plant Extracts Bioactivity

The leaf extracts of *V. amygdalina and C. macrostachyus* demonstrated dose-dependent antibacterial activity against *S. typhi, E. coli*, *K. pneumoniae*, and *S. aureus*. At 200 mg/mL, *V. amygdalina* produced the largest inhibition zone against S. aureus (16.0 ± 2.0 mm), whereas *C. macrostachyus* showed its highest activity *against S. typhi* (11.5 ± 0.0 mm). Ciprofloxacin (30 µg) produced inhibition zones ranging from 21.0 to 23.0 mm, while the negative control (distilled water) showed no inhibition (Table 2).

Table 1. Antibacterial activity of aqueous extracts of *V. amygdalina* and *C. macrostachyus* bacteria (mean inhibition zone ± SEM, mm)

Plant extract	Dose (mg/mL)	*S .typhi*	*E. coli*	*K. pneumoniae*	*S. aureus*
V (L)	200	10.0 ± 5.0^{b}	10.5 ± 2.5^{c}	10.0 ± 0.50^{b}	16.0 ± 2.0^{c}
V (L)	100	10.5 ± 0.5^{b}	10.5 ± 1.5^{c}	9.6 ± 0.30^{a}	11.8 ± 0.5^{a}
V (L)	50	9.0 ± 1.0^{c}	10.0 ± 0.01^{b}	9.0 ± 0.00^{a}	11.0 ± 0.0^{a}
V (L)	25	9.3 ± 0.3^{c}	9.3 ± 0.3^{b}	8.01 ± 0.52^{c}	10.6 ± 0.0^{b}
C (L)	200	11.5 ± 0.0^{a}	10.01 ± 2.8^{c}	8.7 ± 3.5^{c}	10.0 ± 0.0^{b}
C (L)	100	10.6 ± 0.0^{b}	9.2 ± 0.30^{b}	9.7 ± 0.5^{a}	9.0 ± 0.2^{e}
C (L)	50	10.0 ± 0.0^{b}	9.02 ± 0.00^{b}	9.5 ± 3.5^{a}	9.0 ± 0.0^{e}
C (L)	25	9.3 ± 0.3^{c}	8.6 ± 0.0^{a}	9.0 ± 0.0^{c}	8.5 ± 0.6^{e}
PC	30 µg	22.3 ± 0.3^{d}	23.0 ± 0.30^{d}	21.0 ± 0.5^{d}	22.0 ± 0.50^{d}
NC	water	NI	NI	NI	NI

Footnote: V (L) = leaves of *Vernonia amygdalina*; C (L) = leaves of *Croton macrostachyus*
PC = positive control (Ciprofloxacin, 30 µg); NC = negative control (distilled water, 5%)
NI = without inhibition Values are expressed as mean ± SEM (n = 3).
Non similar letter across columun are significant at ($p < 0.05$).

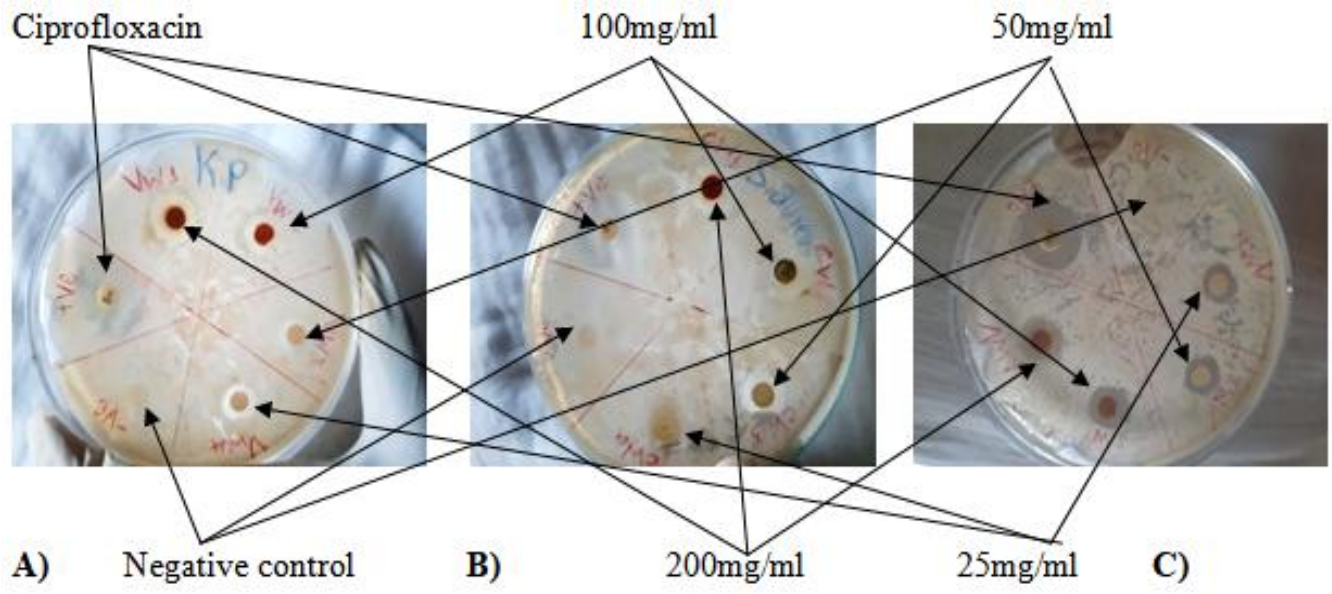

Figure 1 Iinhibition zone of aqueous extract

A) *Vernonia amygdalia* against *K. pnemoniae* (B) *Croton macrostachyus* against *S. aureus* (C) *Vernonia amygdalia* against *S. aureus.*

3.4 Methanol extracts of *Vernonia amygdalia* and *Croton macrostachyus*

Antibacterial activity of both extracts increased with increasing concentration against bacteria growth (Table 3). At 200 mg/mL, *V. amygdalina* showed highest inhibition on *S. aureus* (19.5 ± 1.5 mm), followed by *S. typhi* (14.5 ± 1.5 mm), while *C. macrostachyus* showed its highest activity against *S. aureus* (18.0 ± 0.0 mm).

Table 2 Antibacterial activity of methanolic extracts of the two plants against selected bacterial pathogens (mean inhibition zone ± SEM, mm

Plant extract	Dose (mg/mL)	*S. Typhi*	*E . coli*	*K. pneumoniae*	*S. aureus*
V (L)	200	14.5 ± 1.5^{c}	13.0 ± 0.0^{b}	11.02 ± 0.4^{c}	19.5 ± 1.5^{a}
V (L)	100	12.0 ± 0.12^{b}	11.6 ± 0.3^{a}	10.6 ± 0.12^{a}	12.5 ± 1.51^{b}
V (L)	50	11.03 ± 2.04^{b}	10.5 ± 0.1^{c}	9.0 ± 0.61^{c}	11.06 ± 1.08^{c}
V (L)	25	10.0 ± 0.0^{a}	9.0 ± 0.0^{c}	8.6 ± 0.0^{d}	11.17 ± 0.08^{c}
C (L)	200	11.0 ± 0.57^{b}	13.0 ± 0.57^{b}	12.0 ± 0.0^{c}	18.0 ± 0.07^{a}
C (L)	100	9.04 ± 0.3^{a}	11.0 ± 0.0^{a}	10.05 ± 0.12^{a}	15.02 ± 0.05^{b}
C (L)	50	9.03 ± 0.05^{a}	10.0 ± 0.3^{c}	10.02 ± 0.02^{a}	11.37 ± 0.21^{c}
C (L)	25	8.11 ± 0.15^{e}	9.5 ± 0.0^{c}	9.04 ± 0.07^{d}	10.14 ± 0.15^{e}
PC (Ciprofloxacin 30 µg)	—	22.3 ± 0.58^{d}	21.3 ± 0.33^{d}	23.01 ± 1.15^{e}	24.3 ± 0.35^{d}
NC (Distilled water	—	NI	NI	NI	NI

Footnotes: V (L) = Leaves of *Vernonia amygdalina,* C(L) = Leaves of *Croton macrostachyus*
PC = Ciprofloxacin and NC = distilled water, NI = without inhibition zone. Different letters acroos column are significantly differ at $p \leq 0.05$.

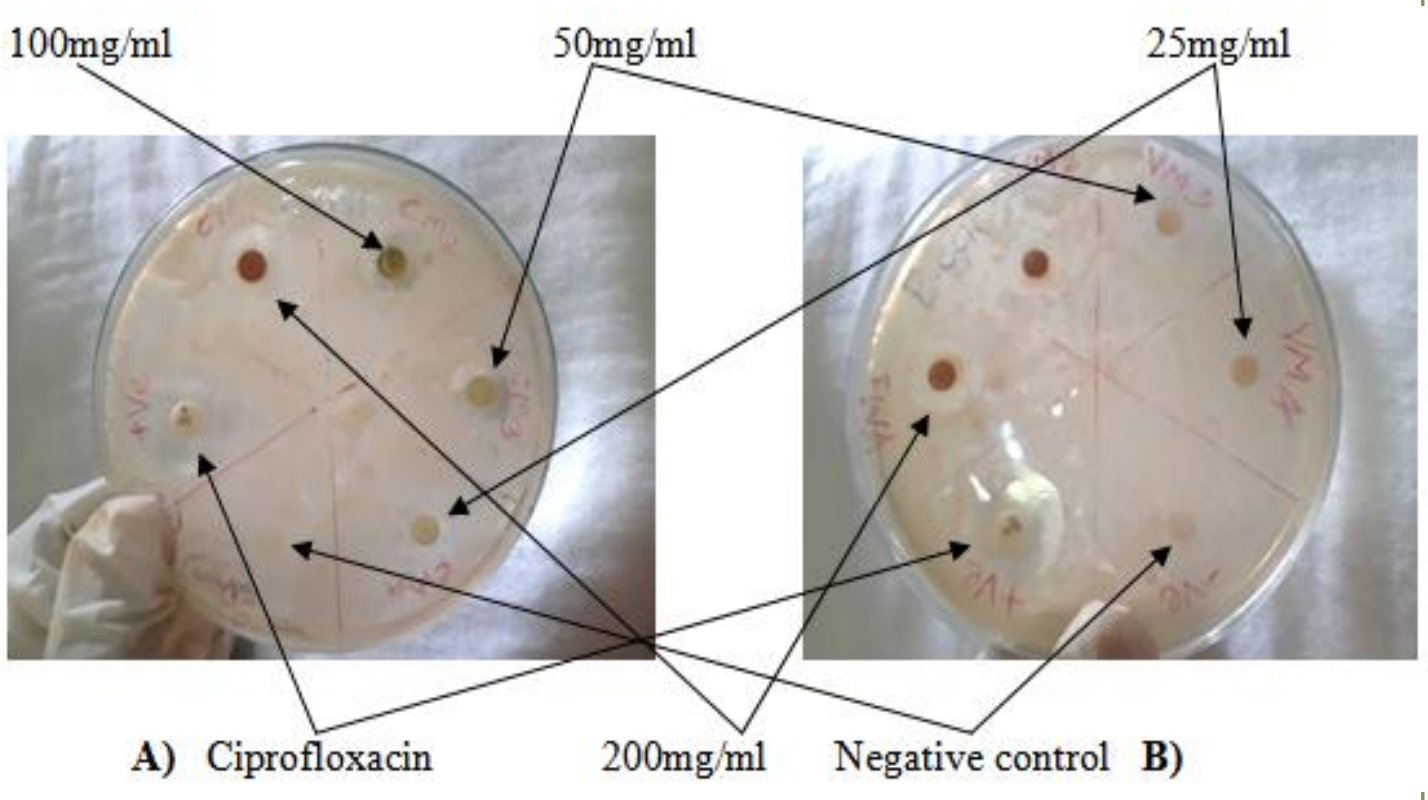

Figure 2 Inhibition zone of Methanol extract of *C. macrostachyus*

A) Methanol extract of *Croton macrostachyus* against *E. coli.* B) Methanol extract of *Vernonia amygdalia* against *E. coli.*

3.5 Determination of MIC

The methanol leaf extract of *V. amygdalina* exhibited MIC MBC at 6.25 and at 12.5 mg/mL against *S. aureus*, respectively. While the extract of *C. macrostachyus* showed MIC and MBC at 12.5 mg/mL against the same organism (Table 4).

Table 3 MIC and MB of *V. amygdalina* and *C. macrostachyus* extracts against selected pathogenic bacteria (mg/mL)

Extract	*E. coli*		*S. typhi*		*S. aureus*		*K. pneumoniae*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
CM	25	50	25	50	12.5	25	25	50
CA	—	—	100	100	50	100	—	—
VM	12.5	25	12.5	25	6.25	12.5	12.5	25
VA	25	50	25	25	12.5	25	25	

Footnote: VM= *V. amygdalia* methanolic extract VA= *V. amygdalia* aqueous extract CM= *C. macrostachyus* methanolic extract CA= *C. macrostachyus* aqueous extract

3.6 Phytochemical Analysis *V. amygdalia* and *C. macrostachyus*

Secondary metabolites including flavonoids and Alkaloids were detected in all plant extracts tested. Saponins were present in both solvent extracts of *C. macrostachyus* and only in the methanol extract of *V. amygdalina*. Tannins were detected in all extracts except the aqueous extract of *C. macrostachyus*. Phenols and terpenes were observed only in the methanol extract of *V. amygdalina,* while they were absent in the extracts of *C. macrostachyus*. Overall, methanol extracts showed a wider range of phytochemical constituents compared to aqueous extracts (Table 5).

Table 4 Phytochemical constituents of methanolic and aqueous extracts of *Croton macrostachyus*

Phytochemicals	*V. amygdalina*		*C. macrostachyus*	
	Extract in methanol	Extracts in water	Extract in methanol	Extracts in water
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	−	+	+
Tannins	+	+	+	−
Phenols	+	+	−	−
Terpenes	+	−	−	−

Footnote: + = phytochemicals present in leaf extract, – = phytochemicals absent in leaf extract

4. Discussions

The present study evaluated antibacterial activities of two indigenous Ethiopian medicinal plants, *V. amygdalina* and *C. macrostachyus*. Among the extracts tested, the highest yield (18.6%) was obtained from the methanolic extract of *V. amygdalina.* In contrast to this, the lowest yield

(10.4%) was recorded from the aqueous extract of *C. macrostachyus.* A previous study reported a lower extraction yield (13%) from the 80% methanolic leaf extract of *V. amygdalina* [17].

V. amygdalina was found to have antibacterial activities on both solvents. However, methanolic extract exhibited greater antibacterial activity than the aqueous extract. This may be due to the higher dissolving ability of methanol to extract bioactive compounds compared with water. The crude extracts of two plants demonstrated considerable antibacterial potentials against four bacteria. These inhibitory effects may be associated with the presence of tannins, alkaloids, flavonoids, saponins, phenols, and terpenes [18].

In the present study, methanolic extracts produced greater inhibition zones than aqueous extracts. This finding is consistent with a previous study reported by [19]. In addition, the methanolic extracts of both plants demonstrated significantly higher inhibitory activity compared with the negative control at all tested concentrations. This may be due to the presence secondary metabolites such as, terpenes, flavonoids, phenols, alkaloids, tannins, and saponins, [20]. The presence of flavonoids, terpenes and phenolic compounds disrupts bacterial cell wall and cytoplasmic membrane integrity and permeability [21]. Tannin was known inhibit the bacterial growth by precipitating cell wall proteins through chelating essential metal ions [22]. Alkaloids inhibit bacterial DNA replication, RNA transcription, and protein synthesis [23].

All aqueous and methanol extracts demonstrated significantly higher inhibitory activity at concentrations of 200, 100, 50, and 25 mg/mL against *S. aureus* compared with *E. coli*, *S. typhi*, and *K. pneumoniae* ($p < 0.05$). This difference may be the structural characteristics of Gram-negative bacteria, which possess a higher permeability barrier to many antimicrobial agents than Gram-positive bacteria [24]. This was supported by the previous study exhibiting higher MIC and MBC values than Gram-positive bacteria [16].

Methalonic extract of *V. amygdalina* showed the highest inhibition zone (19.5 ± 1.5 mm) at 200 mg/mL against *S. aureus*. In contrast, lowest antibacterial inhibition (8.0 ± 0.5) was observed from metabolic extracts of *C. macrostachyus* against *S. typhi* at 25 mg/mL. This suggests that methanolic extracts exhibited greater antibacterial activity than aqueous extracts. Most aqueous extracts showed comparatively weak antibacterial effects against the tested bacterial pathogens

The methanolic extract of *V. amygdalina* showed the lowest MIC and MBC at 6.25 and 12.5 mg/mL against *Staphylococcus aureus*, respectively. This indicates that strong antibacterial activity. This result suggests that methanol is an effective solvent for extracting bioactive compounds with antimicrobial properties. However, MIC values may be influenced by several factors, including incubation temperature, microbial species, inoculum size, and physiological characteristics of the test organisms [25, 26]]. In addition, variations in antibacterial activity may also be affected by plant-related factors such as age of the plant, freshness of plant material, environmental conditions (temperature, light, and water availability), harvesting time, drying method prior to extraction, and the specific plant part used. In contrast, *E.coli* and *K. pneumoniae* did not show MIC and MBC to *C. macrostachyus* aqueous extract at concentration of 50 mg/mL and below it. This may be attributed to the development of resistance strains [26, 27].

In the present study, the common secondary metabolites were screened which could be the attributions for the antibacterial potentials of the plant. Similarly, the previous study revealed that flavonoids and phenolic compounds inhibit the microbial growth by distributing bacterial enzymes [28]. Likewise, tannins disrupt bacterial cell wall [29]. The previous study revealed that most pathogenic bacteria are currently used antibiotics [30]. This challenged our health care system. Therefore medicinal plants have been widely reported as important sources of antimicrobial agents [31].

6. Conclusion

The present study showed that both *V. amygdalina* and *C. macrostachyus* plant extracts possess potential antibacterial activity against selected pathogenic bacteria. Similarly, the two plants contain secondary metabolites, suggesting *V. amygdalina* and *C. macrostachyus* may be promising plant candidate in the control of pathogenic bacteria. Therefore, further

studies are recommended for characterization of these secondary metabolites.

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