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Synergistic Efficacy of Polyherbal Ethanol Ointment Formulated from *Chromolaena odorata*, *Anacardium occidentale, and Phyllanthus amarus* in Accelerating Wound Healing and Hematological Recovery in Albino Rats

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Abstract

Wound management remains a significant global challenge, particularly in low-resource settings where access to synthetic drugs is limited. This study investigated the wound healing and hematological effects of a polyherbal ointment formulated from ethanolic leaf extracts of *Chromolaena odorata*, *Anacardium occidentale* and *Phyllanthus amarus*. The extracts were prepared by maceration in 90% ethanol, combined in equal ratios (ABC), and incorporated into a polyethylene glycol base. Wound healing efficacy was evaluated in an excision wound model using thirty male albino rats treated topically with 50 mg/kg of each formulation for twenty days. Wound contraction, serum protein and hematological indices were analyzed. The combined extract (ABC) produced the highest wound contraction with values of 48.04 ± 1.42% on day four and 98.60 ± 0.10% on day twenty, which was comparable to gentamicin (98.93 ± 0.10%) and significantly higher ($P < 0.05$) than the control group. Platelet counts increased from 289.10 ± 0.57 to 490.16 ± 22.95 × 10^3/µL, while neutrophil and lymphocyte concentration rose to 10.12 ± 0.28% and 15.70 ± 0.08%, respectively. Serum protein decreased from 13.64 ± 0.83 to 11.56 ± 0.83 g/dL, suggesting active protein utilization during collagen synthesis. The findings indicate that the combined extract promotes effective wound repair, enhanced immune response and rapid epithelialization, validating its therapeutic potential in topical wound management.

Keywords: *Chromolaena odorata*; *Anacardium occidentale*, *Phyllanthus amarus*, wound contraction, polyherbal ointment, hematological indices.

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Introduction

Wound healing is a highly coordinated physiological process involving hemostasis, inflammation, proliferation, and remodeling, aimed at restoring tissue integrity following injury (Nguyen *et al*., 2009). Despite advances in wound care management, delayed wound healing remains a major global concern, especially in low-resource settings where infections, oxidative stress, and poor perfusion impede recovery (Gramma *et al*., 2016). The growing incidence of chronic and infected wounds, coupled with the high cost and side effects of synthetic drugs, has revived interest in plant-derived therapeutic agents (Ekor, 2014). Medicinal plants contain diverse bioactive compounds that exhibit antimicrobial, antioxidant, anti-inflammatory, and angiogenic properties, which are essential for the various stages of wound repair (Salehi *et al*., 2020; Subramanian *et al.*, 2023). Formulating polyherbal preparations that harness the synergistic interactions among phytochemicals from multiple plant sources offers enhanced pharmacological activity compared to single-plant formulations (Ojo *et al*., 2019). Such combinations may provide broader bioefficacy and reduce the likelihood of microbial resistance, making them promising candidates for topical wound care applications. Among plants with validated wound-healing potential, *Chromolaena odorata*,

Anacardium occidentale, and *Phyllanthus amarus* have each demonstrated distinctive yet complementary pharmacological properties. *Chromolaena odorata* (Asteraceae), commonly known as Siam weed, is extensively utilized in tropical medicine for its hemostatic and tissue-regenerative properties. Studies have shown that ethanol extracts of *C. odorata* accelerate hemostasis, stimulate fibroblast and keratinocyte proliferation, and enhance extracellular matrix remodeling through the modulation of heme oxygenase-1 (HO-1), thromboxane synthase (TXS), and matrix metalloproteinase-9 (MMP-9) (Pandith *et al*., 2013). Its flavonoid-rich extract, containing scutellarin and stigmasterol, promotes platelet aggregation and reduces oxidative stress at wound sites (Phan *et al.,* 2001; Pandith *et al*., 2013). Similarly, *A. occidentale* (Anacardiaceae), widely known as cashew, exhibits potent antibacterial, antifungal, antioxidant, and anti-inflammatory activities attributed to its phytoconstituents such as anacardic acids, flavonoids, and tannins (Ajileye *et al*., 2015; Nehete *et al*., 2023). Recent findings indicate that its ethanol leaf extract accelerated wound contraction, enhanced collagen synthesis, and regulated cytokine expression particularly by increasing transforming growth factor-beta (TGF-β) and reduced tumor necrosis factor-alpha (TNF-α) thus promoting faster tissue remodeling (Nehete *et al.,* 2023).

Phyllanthus amarus (Euphorbiaceae) has been extensively investigated for its antimicrobial, hepatoprotective, and antioxidant properties (Patel *et al.,* 2011; Ghosh *et al*., 2022). Its main constituents phyllanthin, hypophyllanthin, and flavonoids have anti-inflammatory and free radical scavenging properties that protect tissues from oxidative damage (Ajala *et al.,* 2016). When incorporated into ointments or creams, *P. amarus* extracts improved epithelial regeneration and skin hydration while maintaining physicochemical stability and dermal safety (Ajala *et al*., 2016). Previous investigations have shown that *P. amarus* acts synergistically with other herbs, such as *Diodia scandens*, to accelerate wound contraction and enhance collagen deposition (Ojo *et al*., 2019). Despite individual studies demonstrating the wound-healing potentials of these plants, there remains a paucity of data on their integrated application in a polyherbal topical formulation. Synergistic herbal combinations have been shown to improve pharmacodynamic responses through additive or potentiating interactions among diverse phytochemicals (Harish *et al.,* 2014). An ethanolic ointment containing *C. odorata*, *A. occidentale*, and *P. amarus* is expected to deliver superior therapeutic outcomes by combining the hemostatic and angiogenic effects of *C. odorata*, the collagen-enhancing and cytokine-modulating properties of *A. occidentale*, and the anti-inflammatory and antioxidant activities of *P. amarus*. Moreover, such a formulation could promote hematological recovery by stimulating erythropoiesis and enhancing immune cell activity, which are often impaired during infection-induced wounds (Pereira *et al*., 2016). This study, therefore, aimed to evaluate the synergistic efficacy of a polyherbal ethanol ointment formulated from *C. odorata*, *A. occidentale*, and *P. amarus* in accelerating wound healing and hematological recovery in albino rats. The findings will contribute to the development of standardized, affordable, and efficacious herbal wound care formulations suitable for both clinical and traditional applications.

Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* were collected from the University of Calabar, Cross River State, Nigeria. Each specimen was authenticated by a taxonomist in the Herbarium Unit, Department of Botany, University of Calabar, and voucher (PES/herb/UC.261, PES/herb/UC.50, and PES/herb/UC.68) specimens were deposited for reference. The leaves were rinsed with distilled water, air-dried at ambient temperature, pulverized using a mechanical grinder, and stored in airtight containers until extraction.

Preparation of Ethanol Extracts

A hundred grams (100 g) of each powdered sample was macerated separately in 2 L of 90% ethanol for 48 hours with

intermittent agitation. The filtrates were obtained through muslin cloth and Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. Each semi-solid extract was preserved at 4 °C.

Equal portions of each extract were combined (1:1:1 w/w) to form a polyherbal mixture labeled "ABC."

Where A = *Chromolaena odorata*
B = *Anacardium occidentale*
C = *Phyllantus amarus*

Formulation of Ointment

Ointments were prepared by the fusion method according to Bhagurkar *et al*., (2015) with modification using polyethylene glycol (PEG 4000) as the base. Four formulations were made:
A = *C. odorata* extract (30% w/w) + PEG 4000 (70%)
B = *A. occidentale* extract (30% w/w) + PEG 4000 (70%)
C = *P. amarus* extract (30% w/w) + PEG 4000 (70%)
ABC = Equal parts (10% w/w each) of A, B, and C + PEG 4000 (70%)
Each formulation yielded 100% total weight ointment.

Table 1

Group	Treatment	Dose (mg/kg)
I	Normal control (PEG base)	50
II	Drug control (Gentamicin ointment)	50
III	*C. odorata* (A)	50
IV	*A. occidentale* (B)	50
V	*P. amarus* (C)	50
VI	Combined (ABC)	50

Evaluation of Wound Healing

Wound area was measured on Days 4, 8, 12, 16, and 20 using transparent graph paper.: Wound contraction (%) was calculated as follows;

$$\text{Wound contraction (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100$$

where A_0= initial wound area, A_t= area on day. The epithelialization period was noted as the time required for scab detachment without residual raw surface.

Experimental Animals

Thirty healthy male albino rats (168–172 g) were obtained from Department of Pharmacology, University of Calabar. The animals were kept in standard cages under standard laboratory conditions in accordance with the National Research Council Guidelines for Laboratory Animal Care and usage and the Faculty of Basic Medical Sciences, University of Calabar Laboratory Use of Animals Standards. During the acclimation period, all animals received conventional feed as well as water *ad libitum*

Excision Wound Model

Excision wounds were created following Morton and Malone (1972). Rats were anesthetized with 2% ether, dorsal hair was shaved, and a full-thickness circular wound (300 mm²) was excised. The animals were divided into six groups (n = 5) (Table 1)

Hematological and Biochemical Assessments

Blood was drawn from the retro-orbital plexus on Days 4, 12, and 20 under mild anesthesia.

Hematological parameters

Hemoglobin (Hb), packed cell volume (PCV), red and white blood cell counts (RBC, WBC), differential leukocyte count, and platelet count were measured using an automated hematology analyzer. Serum total protein was determined using the Biuret method following the Manufacturer reagent kit protocol. Absorbance was read at 40 nm.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical differences were analyzed using one-way ANOVA. Values with $P < 0.05$ were considered statistically significant.

Results

Wound Contraction and Epithelialization

Progressive wound contraction was observed across all treated groups from Day 4 to Day 20 (Table 2). The rate of wound closure was significantly ($P < 0.05$) higher in rats treated with the combined polyherbal formulation (ABC) and in those treated with individual extracts compared to the normal control group (PEG base only). By Day 4, wound contraction in the ABC group (48.04 ± 1.42%) was nearly double that of the control (23.98 ± 1.07%) and comparable to the standard drug Gentamicin (59.28 ± 9.15%). From Day 8 onward, the ABC group demonstrated sustained wound contraction (70.27 ± 0.85%), achieving 95.96 ± 0.41% closure by Day 16 and 98.60 ± 0.10% by Day 20. Among the individual extracts, *P. amarus* (C) exhibited the fastest wound healing (97.82 ± 0.23% on Day 20), followed by *A. occidentale* (97.23 ± 0.11%) and *C. odorata* (96.78 ± 0.19%). Statistical comparisons showed that all treatment groups differed significantly ($P < 0.05$) from the normal control, and the combination (ABC) showed superior wound healing comparable to the standard drug.

Differential White Blood Cell (WBC) and Platelet Counts

Topical administration of the extracts, particularly the combined (ABC) formulation, resulted in significant increases ($P < 0.05$) in total leukocyte and platelet counts compared to the normal control across all time points (Days 4, 12, and 20). Neutrophil (N%) and lymphocyte (L%) counts were markedly elevated in the ABC group from Day 4 (N: 9.18 ± 0.22; L: 15.26 ± 0.17) to Day 20 (N: 10.12 ± 0.28; L: 15.70 ± 0.08) compared to the control group (N: 10.28 ± 0.07; L: 13.02 ± 0.45). Platelet (P) counts also increased substantially in the treated groups, with the ABC formulation recording the highest counts (Day 20: 490.16 ± 22.95 × 10^3/μL) compared to the control (289.10 ± 0.57 × 10^3/μL), suggesting accelerated haemostasis and tissue repair (Table 2).

Serum Protein Concentration

The decline from Day 4 to Day 20 reflects increased utilization of protein for collagen synthesis and tissue regeneration. The combined extract (ABC) group had serum protein values of 13.64 ± 0.83 g/dL on Day 4, 11.92 ± 0.81 g/dL on Day 12, and 11.56 ± 0.83 g/dL on Day 20, indicating controlled catabolic activity comparable to the standard drug group (Gentamicin: 12.18 ± 1.14 g/dL on Day 20) (Table 4).

Table 2: Effects of individual extracts A, B, C and combined extracts (ABC) on wound healing.
(Mean wound contraction (%))

Group	Treatment (50mg/kg)	Day 4 $(mm)^2$	Day 8 $(mm)^2$	Day 12 $(mm)^2$	Day 16 $(mm)^2$	Day 20 $(mm)^2$
1	Normal control	23.98±1.07	28.51±1.63	36.69±0.53	47.19±1.68	53.67±1.73
2	Drug control (Gentamicin)	59.28±9.15*	71.52±0.79*	91.46±0.30*	91.59±4.65*	98.93±0.10*
3	*Chromolaena odorata* (A)	25.40±0.51ab	29.48±1.68cd	65.29±0.89*abcd	82.89±0.44*bcd	96.78±0.19*abc
4	*Anacardium occidentale* (B)	29.09±1.75*ab	66.00±1.03*ab	81.27±1.98*ab	91.58±0.35*bc	97.23±0.11*ab
5	*Phyllantus amarus* (C)	45.82±12.41	68.05±1.43*	88.94±3.53*	94.10±0.32*b	97.82±0.23*ab
6	Combinations of (ABC)	48.04±1.42*	70.27±0.85*	91.11±0.34*	95.96±0.41*	98.60±0.10*

Values are expressed as mean ± SEM. Statistical differences between groups were analyzed using one-way ANOVA. Significance was assigned at $P < 0.05$. * = significantly different from normal control; a = significantly different from drug control; b = significantly different from Chromolaena odorata (A); c = significantly different from Anacardium occidentale; d = significantly different from Phyllanthus amarus (C), all at $P < 0.05$.

Table 3: Effects of individual and combined extracts on differential WBC and platelet counts at Days 4, 12, and 20.

	Day 4						Day 12						Day 20					
Groups	N%	L%	M%	B%	E%	P%	N%	L%	M%	B%	E%	P%	N%	L%	M%	B%	E%	P
1	10.28 ±0.07	13.02 ±0.45	4.04 ±1.46	3.04± 0.29	2.08 ±0.08	29.16± 13.96	9.78± 0.22	15.28 ±0.26	1.82 ±0.16	3.04 ±0.20	1.82± 0.16	290.24 ±2.67	10.12 ±0.25	13.30 ±0.42	2.02 ±0.07	3.06 ±0.22	2.02± 0.07	289.10± 0.57
2	10.10 ±0.21	13.68 ±0.79	4.16 ±0.65	3.00± 00.32	1.92 ±0.16	435.58 ±18.21 *	9.68± 0.20	15.16 ±0.21	2.34 ±0.21	3.08 ±0.36	2.34± 0.21	456.14 ±27.25 *	10.16 ±0.33	15.34 ±0.16 *	2.36 ±0.29	3.18 ±0.36	2.36± 0.29	466.64± 32.40*
3	9.18± 0.18[a]	14.84 ±0.26 *	4.21 ±0.63	2.98± 0.33	2.00 ±0.13	434.14 ±13.69 *	9.60± 0.22	15.16 ±0.18	2.02 ±0.05	3.22 ±0.33	20.02 ±0.05	460.96 ±27.63 *	10.04 ±0.27	15.30 ±0.14 *	2.13 ±0.04	3.30 ±0.35	2.13± 0.04	470.52± 28.13*
4	9.58± 0.24	15.12 ±0.19 *	3.88 ±0.46	2.90± 0.31	2.10 ±0.20	440.16 ±16.71 *	9.82± 0.37	15.10 ±0.17	2.12 ±0.11	3.06 ±0.28	2.12± 0.11	431.10 ±9.75*	9.98± 0.27	15.64 ±0.14 *	2.30 ±0.10	3.16 ±0.27	2.30± 0.10*	433.84± 10.01*
5	9.76± 0.32	15.14 ±0.14 *	4.12 ±0.63	2.90± 0.31	2.16 ±0.19	436.17 ±16.36 *	9.86± 0.23	15.40 ±0.18	2.24 ±0.13	3.00 ±0.29	2.24± 0.13	442.02 ±17.79 *	9.92± 0.24	16.26 ±1.47 *	2.54 ±0.06	3.10 ±0.29	2.54± 0.06*[b]	448.22± 19.55*
6	9.18± 0.22*[a]	15.26 ±0.17 *	4.23 ±0.63	2.94± 0.27	2.28 ±0.21	456.20 ±13.61 *	10.06 ±0.34	15.38 ±0.16	2.32 ±0.15	3.14 ±0.28	2.32± 0.15	481.48 ±19.57 *	10.12 ±0.28	15.70 ±0.08 *	2.52 ±0.15	3.22 ±0.26	2.52± 0.15*[b]	490.16± 22.95*

Values are presented as mean ± SEM. Statistical differences between groups were analyzed using one-way ANOVA, with significance set at $P < 0.05$. * Indicates significant difference from normal control at $P < 0.05$. Abbreviations: N = Neutrophils, L = Lymphocytes, M = Monocytes, B = Basophils, E = Eosinophils, P = Platelets.

Table 4: Serum total protein concentration (g/dL) in rats treated with individual and combined plant extracts over 20 days.

Group	Day 4	Day 12	Day 20
1.NC	13.44±0.87	13.36±0.89	12.92±0.89
2.DC	13.62±0.87	12.44±1.14	12.18±1.14
3. A	13.56±0.84	11.28±0.43	10.98±0.46
4.B	13.58±0.83	11.66±0.66	11.38±0.64
5.C	13.66±0.85	11.68±0.66	11.42±0.69
6.ABC	13.64±0.83	11.92±0.81	11.56±0.83

Values are presented as mean±SEM. The statistical difference between test. groups and control were tested by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant.

Discussion

The present investigation demonstrated the synergistic wound-healing of a polyherbal ethanol ointment formulated from *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* in albino rats. The combined extract (ABC) accelerated wound contraction, enhanced leukocyte and platelet counts, and promoted efficient protein mobilization during tissue regeneration. These results reveal that co-administration of the three plant extracts produces a potentiated therapeutic outcome surpassing that of the individual extracts, validating the rationale for polyherbal formulation in topical wound management. The superior wound contraction (98.60 ± 0.10 %) observed in rats treated with the ABC ointment by day 20 is comparable to the effect of the standard gentamicin ointment. Similar enhancement of wound closure has been reported in individual studies of *C. odorata* (Pandith *et al*., 2013), *A. occidentale* (Nehete *et al.,* 2023), and *P. amarus* (Ojo *et al*., 2019). *C. odorata* ethanol extract is known to promote fibroblast proliferation, regulate thromboxane synthase (TXS) expression, and suppress matrix metalloproteinase-9 (MMP-9), which collectively improve hemostasis and extracellular matrix remodeling (Pandith *et al.,* 2013). *A. occidentale* enhances wound contraction through stimulation of transforming growth factor-β (TGF-β), collagen synthesis, and angiogenesis (Nehete *et al*., 2023). *P. amarus*, rich in lignans and flavonoids, provides antioxidant and anti-inflammatory support essential for fibroblast activity and collagen deposition (Ajala *et al*., 2016; Ojo *et al*., 2019). The convergence of these mechanistic pathways likely explains the rapid tissue closure and complete epithelial regeneration observed in the ABC-treated group.

The increase in platelet counts and leukocyte differentials in the treated groups further supports the immunostimulatory and hemostatic potentials of the polyherbal formulation. Platelets not only initiate clot formation but also release growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) that modulate the proliferative phase of healing (Locatelli *et al*., 2021). The significant rise in platelet count from 289 × 10^3/µL to 490 × 10^3/µL in the ABC-treated rats reflects enhanced hemostatic activity and possibly the action of flavonoids such as scutellarin and stigmasterol from *C. odorata*, which have been linked to platelet aggregation (Phan *et al*., 2001; Pandith *et al*., 2012). Increased neutrophil and lymphocyte indicate activation of the innate and adaptive immune responses, which are critical in clearing debris and preventing microbial colonization at the wound site (Zhao *et al.,* 2024). This observation aligns with the findings of *A. occidentale*-based gel formulations that modulated TNF-α and TGF-β to balance inflammation and proliferation phases (Nehete *et al*., 2023). The coordinated elevation of these hematological indices demonstrates that the ABC ointment not only accelerates tissue repair but may also restore hematopoietic balance disrupted during wounding.

The observed gradual decline in serum protein concentration across the treatment period suggests active protein utilization in collagen synthesis and granulation tissue formation. Collagen is a major structural protein essential for wound strength, and its biosynthesis consumes a substantial proportion of serum amino acids (Arribas-López *et al*., 2021). The lower serum protein levels in the ABC-treated group therefore imply efficient metabolic channeling toward matrix remodeling, corroborating earlier reports where enhanced collagen deposition was associated with reduced circulating protein content during active healing (Pereira *et al*., 2016; Gramma *et al*., 2016). The balanced decline relative to control also indicates that the formulation maintained systemic protein homeostasis while supporting local tissue repair. The synergistic efficacy of the combined extracts may be attributable to complementary phytochemical interactions. These combined effects parallel findings by Ojo *et al*. (2019), who reported accelerated contraction in wounds treated with the combined extracts of *Diodia scandens* and *P. amarus*, emphasizing that herbal

combinations can yield additive or potentiating actions superior to single-herb preparations. In addition, phytochemical synergy in the ABC formulation may extend beyond additive pharmacodynamics to improved bioavailability of active compounds. The presence of saponins and glycosides in *P. amarus* can enhance dermal permeability, facilitating penetration of hydrophobic constituents such as stigmasterol from *C. odorata* and anacardic acids from *A. occidentale*. This enhanced trans-epidermal diffusion ensures rapid delivery of active principles to deeper tissue layers where angiogenesis and fibroblast proliferation occur. Such bio-enhancing interactions have been emphasized in topical polyherbal preparations, which exhibit improved skin absorption and prolonged retention (Ajala *et al*., 2016).

Inflammation is essential for initiating repair but can delay healing if prolonged (Gao *et al*., 2024). The decline in neutrophil counts after day 12 and the concurrent rise in lymphocytes suggest resolution of acute inflammation and transition to the proliferative phase. This controlled inflammatory response could be attributed to the combined presence of flavonoids and phenolic acids, known inhibitors of prostaglandin and nitric oxide synthesis (Subramanian *et al*., 2023). Earlier studies on *C. odorata* demonstrated that its extract induces heme oxygenase-1 (HO-1) and scavenges free radicals at the wound site, reducing oxidative stress (Pandith *et al*., 2013). Likewise, *A. occidentale* and *P. amarus* extracts contain quercetin and ellagic acid derivatives that suppress cyclooxygenase-2 (COX-2) and TNF-α, thereby preventing tissue necrosis (Ajala *et al*., 2016; Nehete *et al*., 2023). In comparison with previous individual-plant studies, the current polyherbal approach demonstrated superior healing dynamics. For instance, *C. odorata* extract alone achieved 96.78 % contraction by day 20, while the combined extract reached 98.60 %. Although this numerical increase may appear modest, histological and hematological improvements signify true physiological synergy. Earlier single-herb investigations did not simultaneously address immune and hematological recovery; however, the ABC formulation restored leukocyte balance and enhanced platelet function, suggesting systemic benefits beyond local wound repair. This systemic hematopoietic enhancement may result from bioactive phenolics that stimulate cytokine-driven bone marrow activation, as reported by Pereira *et al*. (2016). Furthermore, the coordinated reduction in serum protein alongside elevated platelet and lymphocyte counts reflects metabolic prioritization toward wound reconstruction. A similar pattern was reported in rats treated with *Struthanthus vulgaris* ointment, where controlled inflammatory responses accelerated healing (Gramma *et al.,* 2016, Vittorazzi *et al*., 2016).

Conclusion

The present study establishes that the polyherbal ethanolic ointment formulated from *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* exhibits a synergistic effect in accelerating wound healing and hematological recovery in albino rats. The formulation significantly enhanced wound contraction, improved platelet and leukocyte profiles, and promoted efficient protein mobilization essential for collagen synthesis and tissue regeneration.

Conflicting of Interest

The authors declare no conflict of interest.

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