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Antibacterial Activity of *Moringa oleifera* Extracts on Bacterial Isolates from Clinical Specimens in a Specialist Hospital, Sokoto

Malami, Hafsat, Dogondaji[1*], Nura Muhammad Bunza[2], Umar, Asiya, Imam[3], Muhammad, Kabiru[2], Tanko Nuhu[3], Muhammad, Yazid, Bukkuyum[2], Sani, Hassan[2], Tambari, Muhammad, Bello[2] and Mikaili, Mainasara, Balkisu[3]

[*1]Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

[2]Department of Medical Microbiology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

[3]Department of Pharmacognozy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding Author:
Malami, Hafsat, Dogondaji

Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

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Abstract:

Background: The rising prevalence of antibiotic resistance among pathogenic bacteria necessitates the exploration of alternative therapeutic agents from medicinal plants. *Moringa oleifera*, widely recognized for its nutritional and medicinal properties, represents a potential source of novel antibacterial compounds.

Objective: This study evaluated the phytochemical composition and antibacterial activity of aqueous and ethanol extracts of *Moringa oleifera* leaves, seeds, and roots against clinical bacterial isolates.

Methods: We collected clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* from the Specialist Hospital in Sokoto. We prepared aqueous and ethanol extracts of *M. oleifera* leaves, seeds, and roots and screened them for phytochemical constituents. We evaluated antibacterial activity using the agar well diffusion method and determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) by broth dilution. We compared the extract's activity with that of ciprofloxacin, a standard antibiotic.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, and carbohydrates, with distribution varying across plant parts and solvents. Ethanol extracts demonstrated stronger antibacterial activity than aqueous extracts. The seed ethanol extract produced the highest zones of inhibition against *E. coli* (25 mm) and *S. aureus* (25 mm). In comparison, the root ethanol extract showed strong activity against *S. aureus* (22 mm) and *S. typhi* (21 mm). *S. aureus* was the most susceptible organism, while *P. aeruginosa* exhibited the least susceptibility. MIC values ranged from 15.6 to 125 mg/mL, and MBC values ranged from 31.3 to 500 mg/mL. Statistical analysis revealed no significant difference in extract activity compared with ciprofloxacin ($p = 0.428$).

Conclusion: *Moringa oleifera* extracts exhibit significant antibacterial activity against clinically relevant bacterial pathogens, supporting their potential as alternative or complementary therapeutic agents. Seed and root extracts showed particular promise, warranting further investigation and development.

Keywords: *Moringa oleifera, antibacterial activity, phytochemicals, clinical isolates, minimum inhibitory concentration, antibiotic resistance*.

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Introduction

The global emergence and spread of antibiotic resistance threaten the effective treatment of common infectious diseases, resulting in increased mortality, disability, and healthcare costs (World Health Organization [WHO], 2015). Antibiotic-resistant strains can withstand most current antibiotics, rendering standard treatments ineffective and increasing the persistence and transmission of infections (WHO, 2015). Beyond resistance, the high cost of conventional antibiotics presents significant challenges in low-income settings, where patients often depend on out-of-pocket payments, leading to incomplete treatment and further resistance development (Okeke et al., 2005). Additionally, toxicity associated with prolonged antibiotic use, particularly hepatotoxicity and nephrotoxicity, along with adverse effects such as gastrointestinal disturbances and allergic reactions, compromises patient compliance and treatment outcomes (Katzung, 2018; Patel et al., 2014).

Moringa oleifera Lam., a member of the Moringaceae family, is a multipurpose tropical tree reaching heights of 10-12 m, primarily used for food with numerous medicinal and industrial applications (Olson et al., 2024). Known locally in northern Nigeria as "Zogale" (Thilza et al., 2010), the plant has been described as a "miracle tree" and a "tree of life" due to its diverse therapeutic properties (Atawodi et al., 2010). Studies have documented its use in treating edema, skin infections, liver damage, stomach disorders, and its potential in cancer prevention (Caceres et al., 2012; Farooq et al., 2007). Its antibacterial, antifungal, and antimicrobial properties may combat infections caused by *Salmonella*, *Rhizopus*, and *Escherichia coli* (Moyo et al., 2012; Mahesh & Satish, 2008).

However, reports on the antimicrobial activity of *M. oleifera* vary considerably. While some studies demonstrate broad-spectrum activity (Rani et al., 2018; Brilhante et al., 2017), others report activity limited to Gram-positive organisms (Kheir et al., 2015), and some suggest mixed, species-dependent efficacy (Marrufo et al., 2013). These variations may arise from differences in harvesting, drying, and preparation methods (Kheir et al., 2015) as well as from geographical factors that affect phytochemical content, including soil type, climate, and storage conditions (Rani et al., 2018).

This study aimed to evaluate the antibacterial activity of *Moringa oleifera* leaf, seed, and root extracts against bacteria isolated from clinical specimens at the Specialist Hospital, Sokoto. Specifically, we investigated the physical and phytochemical composition of the extracts, evaluated their antibacterial activity, determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs), and compared the extracts' activity with that of a standard antibiotic.

Materials and Methods

Study Area and Design

We conducted this comparative *in vitro* experimental study at Specialist Hospital Sokoto, a Sokoto State-owned hospital located in Sokoto metropolis (latitude 13°3′49°N, longitude 5°14′89°E, altitude 272 m). The study employed a comparative *in vitro* experimental design to evaluate phytochemical composition and antibacterial activity of *M. oleifera* extracts against selected bacterial isolates.

Ethical Approval

The Ethical Committee of the Specialist Hospital, Sokoto, granted ethical approval for this study.

Collection and Identification of Plant Material

We collected *Moringa oleifera* plants from the Department of Biological Sciences garden at Usman Danfodiyo University, Sokoto. Dr. Abdulazeez Salihu of the Botany Unit, Department of Biological Sciences, Usman Danfodiyo University, Sokoto, performed taxonomic identification. We deposited a voucher specimen (UDUH/ANS/0999) at the university herbarium.

Preparation of Plant Extracts

We washed leaves, seeds, and roots under running tap water to eliminate dust and other particles, then air-dried them at room temperature for two weeks. We ground the dried materials into a fine powder using a sterile mortar and pestle and stored the powders in dark polythene bags to prevent light exposure.

For aqueous extraction, we dissolved 100 g of each dried, ground powder in 1 L of distilled water for 24 hours. We filtered the mixtures using Whatman No. 1 filter paper and evaporated the filtrates to dryness using a water bath. We collected the extracts in sterile universal bottles and stored them at 4°C until use (Amanze et al., 2020).

For ethanol extraction, we suspended 100 g of each dried, ground powder in 100 mL of 95% ethanol for 24 hours. After filtration using Whatman No. 1 filter paper, we placed the filtrates in an evaporator to remove the solvent and stored them at 4°C (Amanze et al., 2020).

We reconstituted the extracts using sterile distilled water to obtain concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.3 mg/mL, 15.6 mg/mL, 7.8 mg/mL, 3.10 mg/mL, 1.56 mg/mL, and 0.78 mg/mL.

We performed sterility tests by inoculating 1 mL of each extract onto nutrient agar plates, incubating at 37°C for 24 hours, and observing for growth (Cheesebrough, 2006).

Source and Identification of Bacterial Isolates

We collected four bacterial isolates from wound, urine, and blood samples at the Medical Microbiology Laboratory, Specialist Hospital Sokoto: *Staphylococcus aureus* and *Pseudomonas aeruginosa* from wound swab samples, *Escherichia coli* from urine samples, and *Salmonella typhi* from blood samples. We also included reference control strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhi* ATCC 19430.

We subcultured all isolates on nutrient agar plates and incubated them at 37°C for 24 hours to obtain pure cultures. We performed identification through standard morphological, cultural, and biochemical characteristics, including Gram staining, catalase, coagulase, indole, and oxidase tests (Cheesebrough, 2006).

Phytochemical Screening

We screened the extracts for alkaloids, saponins, tannins, flavonoids, glycosides, steroids, phenols, carbohydrates, and anthraquinones using standard methods (El-Sherbiny et al., 2024; Ajayi & Fadeyi, 2015). We tested for tannins by adding 3 drops of 10% ferric chloride to 2 mL of the extract solution; blackish-blue or green coloration indicated the presence of tannins. We tested for saponins by shaking 0.1 g of extract with 5 mL of distilled water; persistent foaming indicated their presence. We tested for alkaloids by adding Dragendorff's reagent to 0.5 g of extract; an orange precipitate indicated the presence of alkaloids. We tested for flavonoids by adding a sodium hydroxide solution to 0.2 g of the extract; as the yellow solution disappeared on acid addition, indicating the presence of flavonoids.

Antibacterial Susceptibility Testing

We evaluated antibacterial activity using the agar well diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012). We prepared Mueller-Hinton agar according to the manufacturer's instructions and poured it into sterile Petri dishes. We prepared bacterial inocula by suspending colonies in nutrient broth and adjusting turbidity to 0.5

McFarland standard (approximately 1.5 × 10^8 CFU/mL) (Cheesebrough, 2003).

We inoculated Mueller-Hinton agar plates with standardized bacterial suspensions using sterile cotton swabs to ensure uniform lawn growth. We aseptically punched 6 mm-diameter wells using a sterile cork borer and filled each well with 30 µL of plant extract at a concentration of 1000 mg/mL. We used sterile distilled water as a negative control and ciprofloxacin (5 µg) as a positive control. We incubated all plates at 37°C for 24 hours and measured zones of inhibition in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

We determined MIC using the broth dilution method (Kawo et al., 2011). We prepared extract concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.3 mg/mL, 15.6 mg/mL, and 7.8 mg/mL by dilution. We mixed 1 mL of each extract concentration with 1 mL Mueller-Hinton broth and added 0.1 mL of standardized inoculum (1.5 × 10^6 CFU/mL). We incubated the tubes at 36°C for 24 hours. We used tubes containing broth and extract as positive controls and tubes containing broth and inocula as negative controls. We recorded the lowest concentration at which no visible growth was observed as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

We determined MBC by subculturing from MIC tubes showing no visible turbidity. Using a sterile loop, we streaked loopfuls from clear MIC tubes onto freshly prepared Mueller-Hinton agar plates. After incubation at 37°C for 24 hours, we recorded the lowest concentration at which no visible bacterial growth was observed as the MBC.

Data Analysis

We analyzed the data using SPSS version 25.0 and presented the results in tables. We compared antibacterial activity between extracts and standard antibiotics using one-way ANOVA, with $p < 0.05$ considered statistically significant.

Results

Physical Properties and Yield of Extracts

Aqueous extraction of *M. oleifera* leaves yielded 17.9 g (17.9%) residue, while ethanol extraction yielded 12.0 g (12.0%). Seed extraction yielded 16.2 g (16.2%) for the aqueous extract and 6.6 g (6.6%) for the ethanol extract. Root extraction yielded 4.7 g (4.7%) for aqueous and 8.3 g (8.3%) for ethanol extracts (Table 1).

Table 1: Physical Properties of *Moringa oleifera* Extracts

Plant Part	Extract Type	Yield (%)	Texture	Colour	Odour
Leaves	Ethanol	12.0	Sticky	Dark green	Sweet
Leaves	Aqueous	17.9	Liquid	Greenish-brown	Leafy
Seeds	Ethanol	6.6	Oily	Light brown	Nutty
Seeds	Aqueous	16.2	Liquid	Light brown	Sweet
Roots	Ethanol	4.7	Sticky	Reddish-brown	Sweet
Roots	Aqueous	8.3	Thick	Brown	Sweet

Phytochemical Composition

Phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, tannins, steroids, glycosides, phenols, and carbohydrates in varying concentrations across extracts, while anthraquinones were absent in all extracts (Table 2). Leaf extracts showed high tannin content and moderate to high flavonoid content. Seed extracts contained saponins, alkaloids, and glycosides. Root extracts demonstrated strong saponin content in the aqueous extract and steroid content in the ethanol extract.

Table 2: Phytochemical Screening Results of *Moringa oleifera* Extracts

Constituent	Method	Leaf Aq	Leaf Eth	Seed Aq	Seed Eth	Root Aq	Root Eth
Saponin	Frothing test	++	+	+	+	+++	-
Alkaloid	Meyer's test	+	+	+	-	+	-
Flavonoids	NaOH test	++	++	+	-	-	+
Tannins	$FeCl_3$ test	+++	++	+	-	-	-

Constituent	Method	Leaf Aq	Leaf Eth	Seed Aq	Seed Eth	Root Aq	Root Eth
Steroid	Liebermann-Buchard's	++	++	+	+	-	+
Glycosides	Keller-Killiani test	+	+	+	+	+	+
Phenols	$FeCl_3$ test	+	+	+	-	-	-
Carbohydrates	Liebermann-Burchard test	+	+	+	+	+	+
Anthraquinones	Bornträger's test	-	-	-	-	-	-

Key: Aq = Aqueous, Eth = Ethanol, + = present, ++ = moderately present, +++ = highly present, - = absent

Antibacterial Activity of Extracts

All extracts demonstrated varying degrees of antibacterial activity against the test organisms, with ethanol extracts generally showing stronger activity than aqueous extracts (Table 3). The seed ethanol extract produced the largest zones of inhibition against *E. coli* (25 mm) and *S. aureus* (25 mm) from the aqueous seed extract. The root ethanol extract showed strong activity against *S. aureus* (22 mm) and *S. typhi* (21 mm). *P. aeruginosa* exhibited the least susceptibility, with several extracts showing no activity against this organism.

Table 3: Antibacterial Activity of *Moringa oleifera* Extracts (Zones of Inhibition in mm)

Extract/Drug	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Leaves aqueous	17	22	0	0
Leaves ethanol	15	7	13	14
Seeds aqueous	0	22	0	10
Seeds ethanol	12	25	12	19
Roots aqueous	15	11	0	0
Roots ethanol	21	22	6	21
Ciprofloxacin	22	30	0	15

The reference control strains (S. aureus ATCC 25923, E. coli ATCC 25922, P. aeruginosa ATCC 27853, and S. typhi ATCC 19430) produced zones of inhibition within acceptable CLSI reference ranges when tested with ciprofloxacin, validating the assay performance.

Minimum Inhibitory Concentration (MIC)

MIC values ranged from 15.6 mg/mL to 125 mg/mL across extracts and organisms (Table 4a). The lowest MIC values (15.6 mg/mL) were observed for leaves ethanol extract against *S. aureus* and *P. aeruginosa*, seeds aqueous extract against *S. aureus*, *E. coli*, and *S. typhi*, and seeds ethanol extract against *E. coli* and *P. aeruginosa*.

Table 4a: Minimum Inhibitory Concentration (MIC) of *M. oleifera* Extracts (mg/mL)

Bacterial Isolate	Leaves Aq	Leaves Eth	Seeds Aq	Seeds Eth	Roots Aq	Roots Eth
S. aureus	62.5	15.6	15.6	31.3	31.3	125
E. coli	125	31.3	15.6	15.6	31.3	62.5
P. aeruginosa	62.5	15.6	125	15.6	125	125
S. typhi	31.3	62.5	15.6	31.3	15.6	15.6

Key: Aq = Aqueous, Eth = Ethanol

Minimum Bactericidal Concentration (MBC)

MBC values ranged from 31.3 mg/mL to 500 mg/mL (Table 4b). The lowest MBC values (31.3 mg/mL) were observed for the leaves' aqueous extract against *S. typhi*, the seeds' aqueous extract against *S. aureus*, *E. coli*, and *S. typhi*, and the roots' ethanol extract against *S. typhi*. The highest MBC (500 mg/mL) was observed for the roots' ethanol extract against *P. aeruginosa*.

Table 4b: Minimum Bactericidal Concentration (MBC) of *M. oleifera* Extracts (mg/mL)

Bacterial Isolate	**Leaves Aq**	**Leaves Eth**	**Seeds Aq**	**Seeds Eth**	**Roots Aq**	**Roots Eth**
S. aureus	125	62.5	31.3	125	125	125
E. coli	250	125	31.3	125	125	250
P. aeruginosa	250	125	250	250	250	500
S. typhi	31.3	125	31.3	125	250	125

Comparison with Standard Antibiotic

Comparison of mean zones of inhibition revealed that seed ethanol extract (17.0 ± 3.1 mm) and root ethanol extract (17.5 ± 3.8 mm) produced mean inhibition zones comparable to ciprofloxacin (16.7 ± 6.4 mm) (Table 5). Statistical analysis using one-way ANOVA revealed no significant difference in antibacterial activity between the extracts and the standard antibiotic ($p = 0.428$).

Table 5: Comparison of Antibacterial Activity with Standard Antibiotic

Extract/Drug	**Mean Zone of Inhibition (mm)**	**p-value**
Leaves aqueous	9.75 ± 5.7	
Leaves ethanol	12.0 ± 1.7	
Seeds aqueous	8.0 ± 5.2	
Seeds ethanol	17.0 ± 3.1	0.428
Roots aqueous	6.5 ± 3.8	
Roots ethanol	17.5 ± 3.8	
Ciprofloxacin (5 µg)	16.7 ± 6.4	

Discussion

The phytochemical composition of *Moringa oleifera* extracts revealed the presence of multiple bioactive secondary metabolites, with distribution varying across plant parts and extraction solvents. This variation aligns with established knowledge that phytochemical content depends on plant tissue type and solvent polarity (Fernández-López et al., 2008). We detected saponins in most extracts, with the highest abundance in the root aqueous extract. Saponins possess membrane-disrupting properties that contribute to antibacterial activity, consistent with reports by Abdullahi et al. (2014) and Anwar et al. (2007). Alkaloids appeared at low concentrations in leaf and seed extracts but were absent in some root extracts; these compounds inhibit bacterial DNA replication and protein synthesis (Sofowora, 2008). Flavonoids, which exert antibacterial effects by binding to bacterial cell walls and extracellular proteins (Cowan, 1999; Mbikay, 2012), were strongly detected in leaf extracts, potentially explaining the observed antibacterial activity. Tannins, abundant in leaf extracts, inactivate microbial enzymes and precipitate cell wall proteins (Doughari et al., 2007). The absence of anthraquinones across all extracts confirms that *M. oleifera* is not a significant source of these compounds (Harborne, 1998).

The varying degrees of antibacterial activity demonstrated by *M. oleifera* extracts against clinical isolates support previous reports of its broad-spectrum antimicrobial properties (Anwar et al., 2007). Ethanol extracts consistently showed stronger activity than aqueous extracts, likely due to ethanol's efficiency in extracting non-polar antibacterial compounds, including flavonoids, phenols, and alkaloids (Sofowora, 2008). Jahan et al. (2022) similarly reported that ethanolic *Moringa* leaf extracts produced inhibition zones of 19-22 mm against *S. aureus* and *E. coli*. However, Kasolo et al. (2010) found comparable activity between aqueous and ethanol extracts, suggesting that solvent efficiency may vary with plant source and extraction protocol.

The marked susceptibility of *Staphylococcus aureus* observed in this study aligns with reports that Gram-positive bacteria are generally more sensitive to plant-derived antimicrobials due to the absence of an outer lipopolysaccharide membrane, which enhances compound permeability (Prescott et al., 2011; Doughari et al., 2007). Conversely, the reduced susceptibility of *Pseudomonas aeruginosa* reflects its intrinsic resistance mechanisms, including effective efflux pump systems (Pandey et al., 2012). However, Nwankwo and Al-Harbi (2014) observed moderate sensitivity of *P. aeruginosa* to ethanol leaf extracts, indicating that strain variation and extract concentration influence susceptibility patterns.

The superior antibacterial activity of root and seed extracts compared to leaf extracts in this study may reflect higher concentrations of potent antimicrobial phytochemicals such as isothiocyanates, alkaloids, saponins, and pterygospermin in these protective organs (Rahman et al., 2009; Cáceres et al., 1991). Abalaka et al. (2012) and Walter et al. (2011) similarly reported superior activity of seed and root extracts. However, contrasting studies have reported higher leaf extract activity linked to flavonoid and tannin content (Bukar et al., 2014; Doughari et al., 2007), highlighting the influence of extraction methods, plant maturity, geographical origin, and bacterial strains on antimicrobial efficacy.

The MIC and MBC results confirm dose-dependent antibacterial activity, with ethanol extracts generally showing lower values indicative of higher potency. This finding aligns with Anwar et al. (2007) and Rahman et al. (2009), who reported lower MICs for ethanol extracts due to improved extraction of phenolics and flavonoids. The higher MIC and MBC values against *P. aeruginosa* align with Cowan's (1999) observation of intrinsic resistance in this organism. However, Bukola et al. (2014) reported stronger activity of aqueous seed extracts with lower MIC values, attributing this to water-soluble bioactive peptides, while Doughari et al. (2007) found comparable MIC values between aqueous and ethanol root extracts, underscoring the variability in antimicrobial activity across studies.

The comparison with a standard antibiotic revealed that root and seed extracts produced zones of inhibition comparable to or exceeding those of ciprofloxacin against certain isolates. This enhanced activity may result from the synergistic action of multiple bioactive compounds targeting multiple bacterial sites simultaneously, unlike conventional antibiotics, which have single modes of action (Chuang et al., 2007; Walter et al., 2011). Rahman et al. (2009) and Abalaka et al. (2012) similarly observed *that Moringa* extract activity was comparable to that of standard antibiotics. However, Doughari et al. (2007) and Bukar et al. (2014) reported superior antibiotic activity, reflecting differences in compound purity, defined dosage, and diffusion properties. The statistical finding of no significant difference in extract and antibiotic activity ($p = 0.428$) supports the potential of *M. oleifera* extracts as complementary antibacterial agents, particularly given their crude preparation. Fahey (2005) emphasized that plant extracts may serve as valuable sources of novel antimicrobial compounds that could be isolated and optimized for enhanced efficacy.

Conclusion

This study demonstrates that *Moringa oleifera* leaves, seeds, and roots contain bioactive phytochemicals, including alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and steroids, which confer significant antibacterial activity against clinically relevant pathogens. Ethanol extracts were more effective than aqueous extracts, with seed and root extracts showing particular promise. *Staphylococcus aureus* was the most susceptible organism, while *Pseudomonas aeruginosa* demonstrated the least susceptibility. The MIC and MBC results confirmed dose-dependent bactericidal activity. Importantly, the extracts showed activity comparable to that of ciprofloxacin against certain isolates, supporting their potential as alternative or complementary therapeutic agents.

Recommendations

Based on these findings, we recommend:

1. Isolation and purification of individual phytochemical constituents to identify specific compounds responsible for antibacterial activity and determine their relative potency.
2. Development of seed extracts into topical formulations, such as creams and ointments, for potential clinical applications.

3. Exploration of additional solvents, including methanol, chloroform, and ethyl acetate, to compare extraction efficiency and antibacterial activity.
4. Further investigation into the mechanisms of action of *M. oleifera* extracts, particularly against resistant bacterial strains.
5. Toxicity studies to establish safety profiles for potential therapeutic applications.

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