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Green Synthesis of Silver Nanoparticles Using *Couroupitaguianensis* Flower Extract and Evaluation of Antibacterial Activities

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Abstract:

The field of bio nanotechnology has arisen as a result of the combination of biotechnology with nanotechnology, with the aim of producing environmentally acceptable technology for the production of nanomaterials by biosynthetic means. The bactericidal effects of silver have been recognised for millennia. In contemporary times, there has been extensive utilisation of silver-based topical dressings as a therapeutic approach for managing infections in cases of burns, open wounds, and chronic ulcers. The present work aims to investigate a green synthesis of AgNPs using *C. guianensis* flower extract as reducing and stabilizing agents. The AgNPs formation was monitored using UV-Vis spectrophotometer. Among the test bacteria, the highest zone of inhibition was found in *Micrococcus luteus* (16.8 ± 0.5) and Pseudomaonas putida (9.2 ± 0.3) and the least was recorded in *Proteus mirabilis* (4.2 ± 0.1) and *Rhodococcusrhodochrous* (4.5 ± 0.3). Hence, the synthesised AgNPs can potentially be applied for water treatment and medicinal purposes.

Key words: Green synthesis, AgNPs, flower extract, antibacterial activity

Introduction:

Nanotechnology has garnered significant interest as a burgeoning field of study focused on the advancement of nanomaterials and nanoparticles (NPs) for use in various domains, including catalysis, electrochemistry, biomedicine, pharmaceuticals, sensors, food technology, and cosmetics [1-3].Nanoparticles, also known as NPs, are solid particles at the atomic or molecular scale that possess exceptional physical properties in comparison to bulk molecules. These properties are contingent upon the size and morphology of the nanoparticles, which typically measure less than 100 nm in diameter [4-6].Metal and metal oxide nanoparticles have been extensively investigated in the field of science and technology due to their exceptional characteristics, including a high surface-to-volume ratio and efficient dispersion in solution. As a result of these factors, metal and metal oxide nanoparticles have heightened antibacterial characteristics [7-9].Nanoparticles can be manufactured by a range of methodologies, encompassing chemical, physical, and biological techniques. The chemical approach of synthesizing nanoparticles is known for its ability to produce vast quantities in a relatively short amount of time. However, this method necessitates the use of capping agents to ensure the size stability of the nanoparticles. The chemicals employed in the synthesis and stabilization of nanoparticles possess hazardous properties and results in the production of environmentally detrimental byproducts. There is a growing interest in biological techniques for nanoparticle synthesis due to the demand for environmentally non-toxic synthetic protocols. These approaches aim to eliminate the usage of harmful chemicals as byproducts.

Consequently, there has been a growing need for the implementation of environmentally-friendly nanotechnology, sometimes referred to as "green nanotechnology." Numerous biological methodologies have been documented to date for the manufacture of nanoparticles, encompassing both extracellular and intracellular synthesis, employing microorganisms such as bacteria. fungi,[10,11]and plants [12-14]Plants offer a more advantageous medium for the synthesis of nanoparticles due to their absence of hazardous substances and their provision of natural capping agents. Additionally, the utilization of plant extracts also serves to decrease the expenditure associated with microorganisms. The utilization of isolation and culture media has been found to enhance the cost competitiveness of synthesizing nanoparticles by microorganisms. Plant tissues possess a diverse array of metabolites that exhibit the capacity to function as both reducing and capping agents during the synthesis of metal nanoparticles. Consequently, they represent a highly promising alternative to conventional methodologies for the large-scale production of nanoparticles. The primary mechanism under consideration for the process is plant-assisted reduction, which is attributed to the presence of phytochemicals [15,16]. The primary phytochemical constituents implicated in this context encompass terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Water-soluble phytochemicals, namely flavones, organic acids, and quinones, play a pivotal role in the prompt reduction of ions. Recent studies have indicated that the utilization of plant extracts for the reduction of metal ions exhibits a significantly higher rate compared to microorganisms. Furthermore, these investigations have observed the creation of colloidal metal nanoparticles at elevated levels[17].

This study was conducted with the objective of investigating the green synthesis of AgNPs utilizing a Flower extract of *Couroupitaguianensis* as both reducing and stabilizing agents, in order to address the existing research gap. An investigation was carried out to study the bio-reduction reaction utilizing the UV-Visible spectroscopy technique. The application involved in the evaluation of the antibacterial properties of AgNPs against both Gram-positive and Gram-negative microorganisms.

Material and Methods:

Plant Material:

Couroupitaguianensis, known by a variety of common names including cannonball tree, is a deciduous tree in the flowering plant family Lecythidaceae.
The classification of *Couroupitaguianensis* is given in the following:
Kingdom: Plantae
Family: Lecythidaceae
Genus: Couroupita
Species: *C. guianensis*
Common Name: Cannonball tree

Synthesis of Silver Nanoparticles

$AgNO_3$ is the most commonly used metal precursor in the synthesis of AgNPs. PFE (Plant Flower Extract) solution was mixed with $AgNO_3$ solution of 1 mM (100 ml), which pre-conditioned to the set parameters. Immediate color change from light green to brown color was observed, indicating the ultra-fast synthesis of AgNPs in the reaction mixture.

Ultraviolet-visible Spectra Analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

Antibacterial activity of AgNPs

The agar well-diffusion method was used to investigate the antibacterial properties of Plant Flower mediated silver nanoparticles made by *C. guianensis* utilizing various types of Gram-positive and Gram-negative bacteria. The nutrient agar medium was placed into sterile petri dishes in an amount of around 20 ml. The test bacteria were cultured in nutritional broth for 24 hours. Bacterial lawns were created using a 100µl nutrient broth culture of each bacterial species (1×10^5 CFU/ml). With the use of a sterile stainless steel cork borer, 8 mm diameter agar wells were created. A positive control of 60µl of 30 g/ml streptomycin was added to the wells along with 60µl of plant flower extract solution, 60µl of 1mM silver nitrate, and 60µl of plant flower-mediated silver nanoparticles. Zones of inhibition were checked on the plates after a 24-hour incubation period at 37 °C. For each organism, the mean value of the diameter of these zones of inhibition was recorded and represented in millimeter units.

Results and Discussion:

In the Current study *C. Guianensis* flower extract was used to synthesis silver nanoparticles.The flower extract's bioactive ingredients were crucial in the formation of silver nanoparticles.The formation and stability of AgNPs in an aqueous colloidal solution was investigated by using UV–vis-NIR spectral analysis. As expected, AgNPs turned yellowish brown in the aqueous solution, which has been attributed to the excitation of surface plasmon resonance in AgNPs. The present work reports the occurrence of SPR at 422nm which is similar to the study conducted by Safdar M et al. Figure 1 presents the UV-Vis spectra showing a sharp peak in the characteristic wavelength range associated with the AgNPs. Thus, the initial confirmation of NPs synthesized was AgNPs was provided using UV-Vis spec [18].

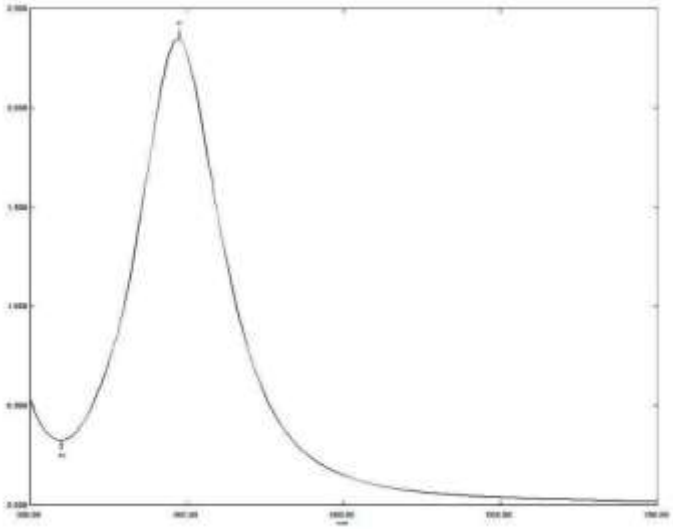

Figure: 1 UV-Visible Spectroscopy of *Couroupita guianensis* mediated silver nanoparticles.

Antimicrobial activity of biologically synthesized silver nanoparticles was studied against 12 pathogenic bacteria in which 6 are gram positive and 6 are gram negative using

agar well diffusion method. The wells were loaded with 60µl of Ag nanoparticles solution, 60µl of 1mM silver nitrate and 60µl of *C. guianensis* flower extract (without AgNo3) as a negative control, along with 60µl of 30 µg ml−1 of streptomycin as a positive control (Figure 2). Among the test bacteria, the highest zone of inhibition was found in *Micrococcus luteus* (16.8 ± 0.5) and *Pseudomaonas putida* (9.2 ± 0.3) and the least was recorded in *Proteus mirabilis* (4.2 ± 0.1) and *Rhodococcus rhodochrous* (4.5 ± 0.3) (Figure 3) (Table 2). A similar study was conducted by Kumar H et al 2020 the study revealed that silver nanoparticles had notable efficacy in suppressing bacterial growth. Among the tested bacteria, *Micrococcus luteus* demonstrated the largest zone of inhibition, measuring at 21.8±0.2 mm, followed by Staphylococcus aureus with a zone of inhibition of 20.2 ± 0.3 mm [19]. The utilisation of the Catharanthus roseus flower in the production of AgNPs has demonstrated promising antibacterial properties against various bacterial strains including *Bacillus subtilis, E. coli, Klebsiella pneumoniae, Pseudomonas putida*, and *S. aureus* [20]. According to the study conducted by Padalia et al. (2014), it was observed that silver nanoparticles (AgNPs) derived from the flower extract of *Tagetes erecta* exhibited a higher level of antibacterial efficacy against *S. aureus* compared to *Bacillus cereus* [21]. According to a study conducted by *Lee et al.* (2019), it was shown that the extract of Tussilago farfara flower buds, which contains sesquiterpenoids, demonstrated high efficacy as a reducing agent in the manufacture of silver nanoparticles (AgNPs) [22].The potential mechanism underlying the antimicrobial efficacy of nanoparticles is believed to involve their uptake by the host organism. Once inside the host, these nanoparticles initiate the generation of free radicals, which disrupt the fundamental metabolic processes and, in certain cases, may induce genetic alterations or damage to the host's genome. Ultimately, this leads to the demise of the host organism [23,24]. Additionally, the findings of this study align with the conclusions of various other researchers who have investigated the creation of silver nanoparticles utilising flower extracts.

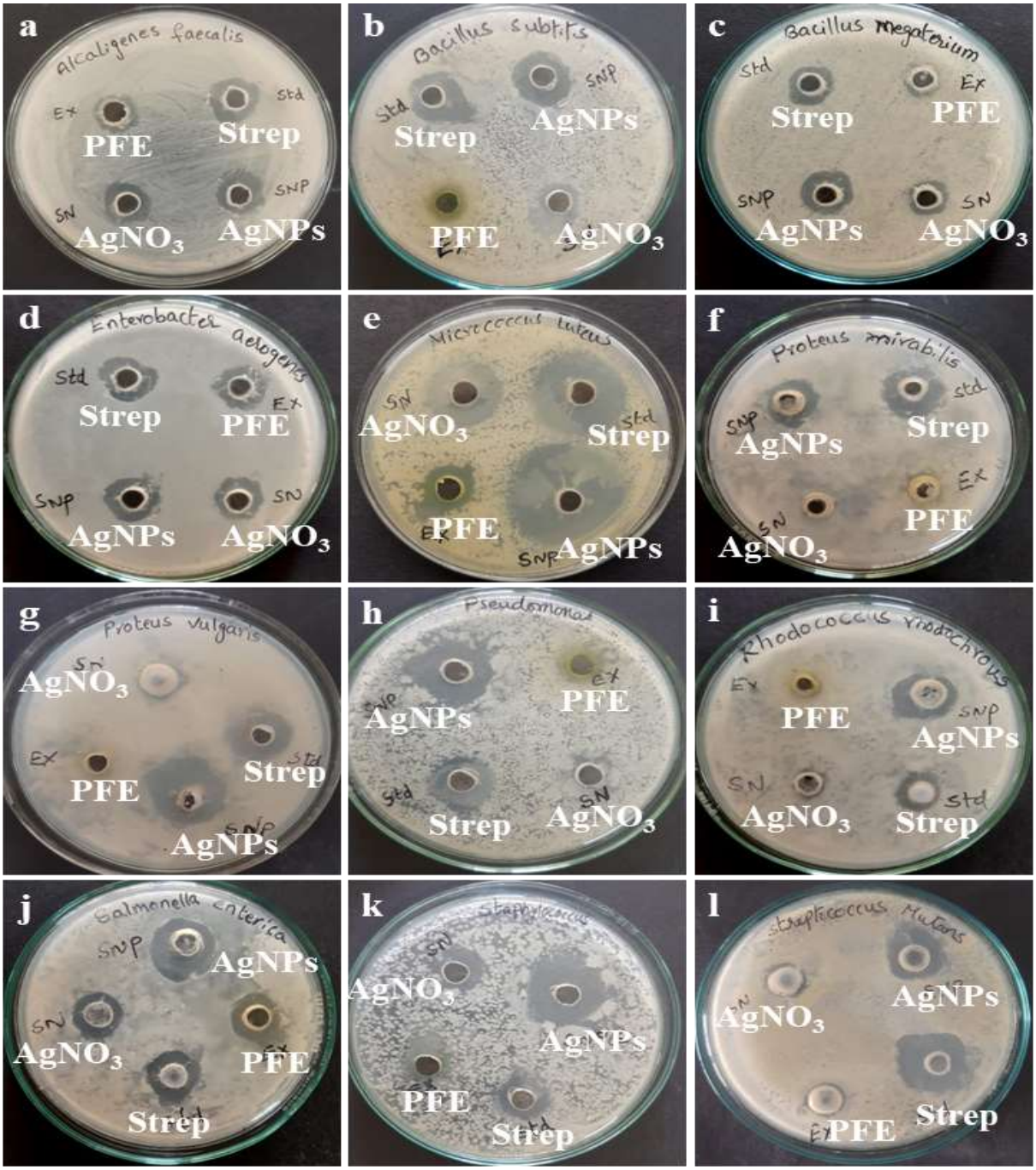

Figure 2: Antibacterial activity via standard agar well diffusion against **(a)***A. faecalis* (MTCC 126), **(b)***B. subtilis* (MTCC 441), **(c)***B. megaterium* (MTCC 428), **(d)***E. aerogenes* (MTCC 10208), **(e)***M. luteus* (MTCC 106), **(f)***P. mirabilis* (MTCC 425), **(g)***P. vulgaris* (MTCC 426), **(h)***P. aeruginosa* (MTCC 1688), **(i)***R. rhodochrous* (MTCC 265), **(j)***S. enterica* (MTCC 3858), **(k)***S. aureus* (MTCC 737), and **(l)***S. mutans* (MTCC 497) with $AgNO_3$ (Negative control), Streptomycin (Positive control; Commercial standard), PFE, and AgNPs.

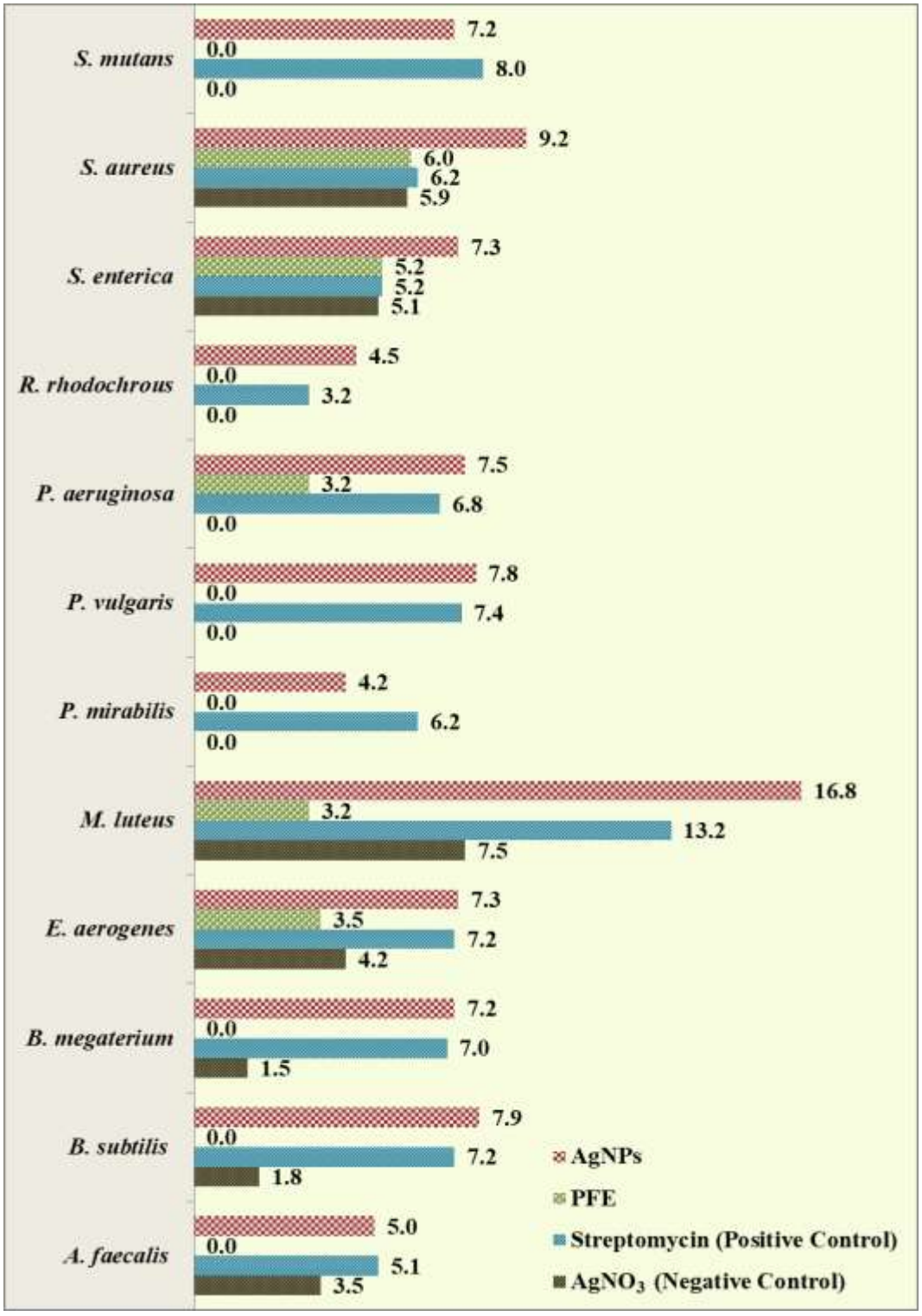

Figure 03: Bar graph showing the ZOI (in mm) of the controls and test samples against the test bacteria.

Table 1: Antibacterial activity (ZOI in mm) of controls and test samples against the test bacteria.

Test Bacteria	Control(s)		Test Sample(s)	
	$AgNO_3$ (Negative)	Streptomycin (Positive)	PFE	AgNPs
Alcaligens faecalis	3.5 ± 0.5	5.1 ± 0.2	-	5.0 ± 0.1
Bacillus subtilis	1.8 ± 0.5	7.2 ± 0.5	-	7.9 ± 0.2
Bacillus megaterium	1.5 ± 0.5	7.0 ± 0.2	-	7.2 ± 0.1
Enterobacter aerogenes	4.2 ± 0.2	7.2 ± 0.3	3.5 ± 0.2	7.3 ± 0.1
Micrococcus luteus	7.5 ± 0.3	13.2 ± 0.2	3.2 ± 0.1	16.8 ± 0.5
Proteus mirabilis	-	6.2 ± 0.2	-	4.2 ± 0.1
Proteus vulgaris	-	7.4 ± 0.3	-	7.8 ± 0.3
Pseudomonas aeruginosa	-	6.8 ± 0.5	3.2 ± 0.3	7.5 ± 0.3
Rhodococcusrhodochrous	-	3.2 ± 0.3	-	4.5 ± 0.3
Salmonella enterica	5.1 ± 0.5	5.2 ± 0.2	5.2 ± 0.3	7.3 ± 0.2
Staphylococcus aureus	5.9 ± 0.3	6.2 ± 0.2	6.0 ± 0.1	9.2 ± 0.3
Streptococcus mutans	-	8.0 ± 0.2	-	7.2 ± 0.1

Conclusion:

The subject of nanotechnology is currently faced with a significant imperative, namely the requirement for the establishment of a dependable and environmentally sustainable method for the production of metallic nanoparticles. Nanoparticles are widely regarded as the essential constituents of nanotechnology. The utilisation of silver nanoparticles in the domains of biology and medicine is of significant importance owing to their appealing physiochemical characteristics. In the current investigation, we have successfully illustrated the ability of a cost-effective, naturally occurring biological reducing agent, in conjunction with extracts derived from *C. guianensis* flower, to generate metal nanostructures. This

process employs an efficient green nanochemistry technology, thereby circumventing the need for harmful solvents and minimising waste production. The current investigation demonstrated a straight forward, expeditious, and cost-effective method for the production of silver nanoparticles. The utilisation of *C. guianensis* possesses the additional benefit of being applicable in nanotechnology processing sectors. The utilisation of prepared nanoparticles exhibits potential in various fields such as antibacterial applications, wound healing, water purification, and medicine. Consequently, this technology has promise for the large-scale synthesis of nanoparticles.

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