Green Biosynthesis and Bioactivity Assessment of AgNPs and NaNPs from *Pycnanthus angolensis* Sap: Experimental and Computational Validation of Ethnomedicinal Use

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Abstract

Background: Nanoparticles are materials with at least one dimension ranging between one and 100 nanometers, and at this scale, they display unique physical, chemical, and biological properties distinct from their bulk counterparts. **Objective**: To synthesise and characterise silver and sodium nanoparticles from plant sap, evaluate their antimicrobial and antioxidant properties, and provide scientific validation for the traditional use of sodium chloride in herbal cough remedies. **Methodology**: In this study, Silver (AgNPS) and sodium (NaNPS) nanoparticles were synthesised and characterised using ultraviolet (UV), Fourier Transform Infrared (FT-IR) spectroscopy, and gas chromatography–mass spectrometry (GC-MS). Their biological activities were evaluated against *Staphylococcus aureus* and *Aspergillus niger*, while antioxidant potential was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Density Functional Theory (DFT) calculations at the B3LYP/6-31G level were employed to estimate the HOMO–LUMO energy gap and related global reactivity parameters. **Results**: Two major compounds were identified: 2-(4-hydroxy-3-methoxyphenyl)propanamide (73.05%) and 4,4'-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) (26.95%). Antimicrobial screening revealed inhibition zones of 19 mm for NaNPS and 10 mm for AgNPS against *S. aureus*. Radical scavenging activity followed the trend NaNPS > crude sap > AgNPS. Theoretical studies indicated that 4,4'-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) is more potent than the major constituent, 2-(4-hydroxy-3-methoxyphenyl)propanamide. **Conclusion/Recommendations**: The findings provide scientific justification for the traditional use of sodium chloride (NaCl, common salt) in the preparation of this plant's sap for cough remedies. Given the superior bioactivity of the minor compound 4,4'-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) predicted by DFT calculations, isolation and structure-activity relationship studies of this compound should be prioritised to develop more potent antimicrobial and antioxidant agents for pharmaceutical applications.

Keywords: *Pycnanthus angolensis* sap, Na and Ag Nanoparticles, cough management, Herbal medicine.

Introduction

The drugs of modern society are products of intensive research and technological advancement, with many of their raw materials being natural, gifts from plants in the form of phytochemicals (Chinaza *et al.,* 2018). Phytochemicals are plant natural products that possess numerous therapeutic properties. Traditional medicines have utilised the beneficial properties associated with these compounds for centuries, highlighting their potential to become novel drug candidates (Atanasov *et al.,* 2021). Modern scientific approaches, such as structural and computational biology, offer unprecedented opportunities to study these natural products further. Analysis conducted via structural biology techniques has revealed three-dimensional structures of phytochemicals that can aid investigations with

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molecular docking or virtual screening to find new pharmacologically active molecules (Chihomvu *et al.,* 2024). Traditionally, it is believed that the synergy among various active ingredients—secondary metabolites—present in herbal preparations is mainly responsible for their therapeutic benefits (Prajapati *et al.,* 2003). In many developing countries, people still depend heavily on traditional healers and medicinal plants to meet their healthcare needs. Although modern medicine exists alongside traditional practices, herbs have retained popularity due to their deep cultural and historical roots (Briskin, 2000; Aziz *et al.*, 2018; and Keter and Mutiso, 2012).

In recent times, however, some herbal ingredients have been commercialised without due consideration of the traditional healing systems from which they originated (Duru & Onuh, 2018). Regulatory approaches vary across countries: in Germany, for instance, herbal products are marketed as phytomedicines and subjected to rigorous standards of efficacy, safety, and quality comparable to those of conventional drugs. In contrast, in the United States, most herbal products are marketed and regulated as dietary supplements, a category that does not require pre-approval (Cai *et al.,* 2004).

The use of herbal medicines predates civilisation and persists across all societies, regardless of their level of development (Pan *et al.,* 2013). Even today, some plant-derived drugs are obtained through extraction, even though their chemical structures and synthetic production methods are already established. However, the high cost of laboratory synthesis often makes natural extraction more practical and affordable. Plant-based compounds are not only cost-effective but are also generally associated with fewer side effects. Additionally, the continuous emergence of antibiotic-resistant bacteria has intensified the search for novel and more effective therapeutic agents, with plants being a primary focus of exploration (Martinez *et al.,* 2004; Newman & Cragg, 2016).

To contribute to this effort, various parts of *Pycnanthus angolensis* have been studied, and several compounds with significant therapeutic potential have been reported. The present study aims to investigate the sap of *Pycnanthus angolensis*, identify its chemical constituents, synthesise nanoparticles (NaNPs and AgNPs), characterise them, and evaluate their bioactivities—particularly to provide a scientific basis for the traditional practice of adding sodium chloride before using the sap as a cough remedy.

Materials and Methods

Collection and Identification of Sap of *P. angolensis*

The sample was collected into an air-tight bottle at Tijani Adeagbo Estate, Egbedore local government area, Okinnin and was identified at the Department of Botany Herbarium, University of Ibadan. Ibadan, Nigeria.

Biosynthesis of AgNPs and NaNPs

The green synthesis of AgNPs and NaNPs was prepared following the methods reported by Dada *et al*. (2013). Preparation of AgNPs was done by reacting 5 mL of the sap, which acts as a reducing agent, with 10 mL of $AgNO_3$ solution (1 mM) and was homogenised on the magnetic stirrer for 10 min at ambient temperature. A colour change was observed from pink to black. The mixture was centrifuged, and the residue was dried in an oven between 45 ^oC and 50 ^oC. NaNPs was also prepared following the same method, but the crystal was reddish in colour. The concentration of synthesised NaNPS and AgNPS was determined by concentrating a known volume of colloidal NaNPS and AgNPS through high-speed centrifugation (10,000 rpm) and drying at 60 °C, followed by weight determination on a Metler balance. The concentrations of stock NaNPS and AgNPS synthesised were 50 mg/mL and 25 mg/mL, respectively.

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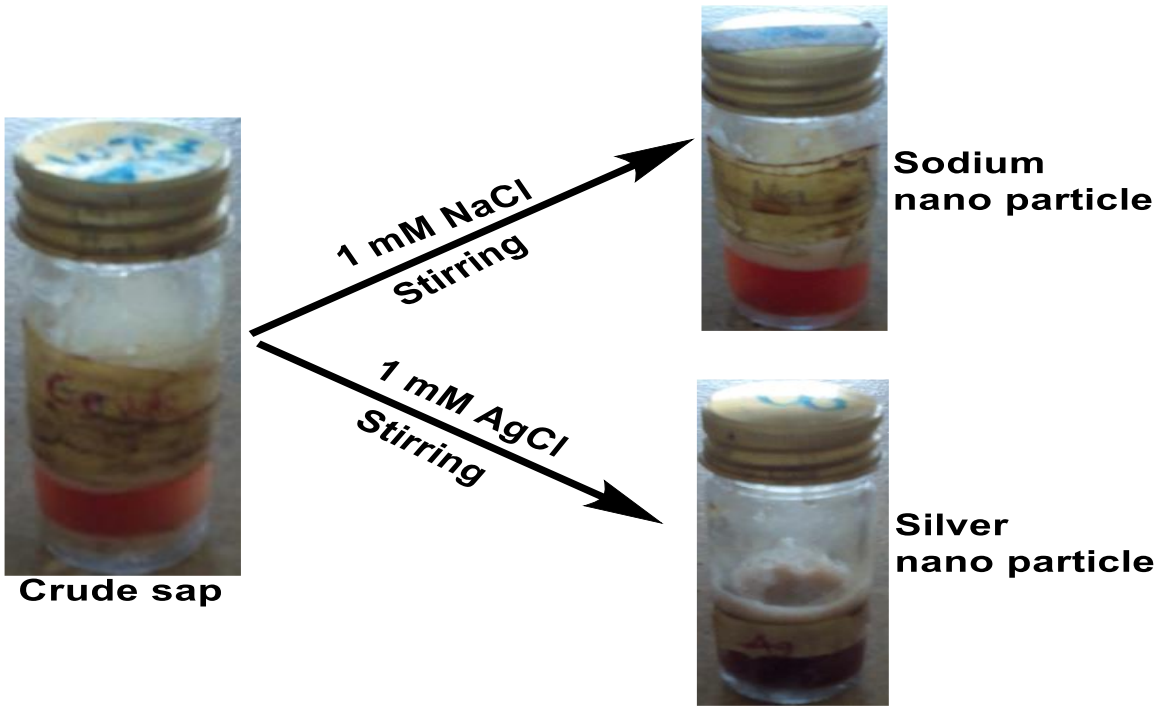

Plate 1: Biosynthesis of nanoparticle

UV-Vis Analysis of the Nano Compounds

The reduction of metallic Ag^+ and Na^+ ions was monitored using a UV-Vis spectrometer. The spectra were obtained by measuring the absorption of the prepared nanoparticle dispersion in a quartz cuvette with a 1 cm optical path. A small aliquot was drawn from the reaction mixture, and a spectrum was taken on a wavelength from 200 nm to 600 nm.

FT-IR Analysis of the Nano Compounds

About 1 mg of each sample (in solid form) was finely ground in a small mortar with pure potassium bromide. The mixture was pressed into a disc using a special mould and a hydraulic press. The functional group was determined using an infrared spectrophotometer (FTIR-820IA single beam laser Shimadzu Infrared Spectrophotometer).

GC-MS Analysis of *Pycnanthus angolensis* sap

The crude sap of *Pycnanthus angolensis* was analysed using an Agilent GC-MS. The gas chromatography Model: 7890A (GC) analyses were performed on an Agilent Technologies interfaced with a J and W DB-35ms column (30 m x 0.25 mm, 0.25 µm) and a Mass Selective Detector Model 300 °C (MSD) transfer line, with a full scan at m/z 50-500. An electron ionisation system with an ionisation energy of 70 eV was used for the detection of compounds. Inert gas helium (99% purity) was used as a carrier gas at a constant flow rate of 35 cm/sec, while HP-5 (30 mm X 0.25 mmX 0.320 µm) was used as the stationary phase. The oven temperature was programmed to start at 60 °C, held for 1 minute, then increased by 4 °C/min to 110 °C for 3 minutes, followed by 8 °C/min to 260 °C for 5 minutes, and finally 10 °C/min to 300 °C for 12 minutes. The crude sample was diluted with an appropriate solvent (1/100, v/v) and filtered. The particle-free diluted sap (1 µl) was taken in a syringe and injected into the injector in split mode. The split ratio was 1:120. The percentage composition of the crude extract constituents was expressed by peak area.

The chemical compounds in the sample were identified and characterised based on GC retention time. The mass spectra were matched with those of standards available in the existing computer library (Mainlib and Tutorial of GC-MS systems) (Alabi *et al.,* 2019).

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SEM Analysis

Samples were prepared for scanning electron microscopy (SEM) by coating them in gold using a Balzers' sputtering device. The samples were viewed using a TESCAN Vega TS 5136LM SEM, typically at 20 kV at a working distance of 20 mm.

Antioxidant activity

The antioxidant activity of the samples and the standard was evaluated as reported by Prieto with slight modification (Prieto *et al.*, 1999). The crude extracts were diluted with appropriate solvents, and serial dilution was prepared: 12.5, 25, 50, 100 and 200 ppm. From each concentration, 4 mL was measured into a test tube. 2,2-diphenyl-1-picrylhydrazyl (DPPH) 1 ml, 0.1 mM in methanol was added to the test tube and shaken vigorously. All the test tubes were allowed to stand in a dark cupboard for 45 minutes at 27 °C after being thoroughly shaken. A control was also set up. The absorbance of the mixture was measured by a UV spectrophotometer (JENWAY 6300 Spectrophotometer) at a wavelength of 517 nm. The total antioxidant activity of the crude extracts was estimated as the inhibition percentage and was calculated by using the standard formula.

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) \div Abs_{control}] \times 100 \quad \text{(eqn 1)}$$

Inhibitory Test

Bacteria and Fungi

The bacterium used for this experiment was *Staphylococcus aureus*. It was cultured aerobically at 37 °C for 24 hours on peptone water. The agar well diffusion method was used (Alabi & Lajide, 2017 and Alabi *et al.,* 2020). The microbe was seeded on the sterile nutrient agar (NA) Plate containing 8 mm wells. About 0.5 ml (0.02 g/ml) of each sample was then introduced into the bored well and incubated for 24 hours at 37 °C.

Control Plates were also set up using Dimethylformamide (D.M.F.), methanol, and the standard antibiotic Streptomycin Sulphate. Zones of inhibition around the wells were measured, and the results were quoted as the radii (mm) of these zones.

The fungus isolate used for this experiment was *Aspergillus niger*. Samples (0.02 g/ml) were prepared, and 0.5 ml of each sample was aseptically mixed with 15 ml of sterile molten potato dextrose agar (PDA). The fungus was inoculated at the centre of the Plate with the aid of a 4 mm cork borer, a sterile needle and a syringe. Benhite, a standard antifungal agent, was used as a control at 2.5 g/ml (D.M.F.), and methanol-impregnated Plate s were prepared in the same manner as a control. All the Plate s were incubated at 27 °C for 72 hours, and mycelial growth was measured at 24-hour intervals. Mycelia growth inhibition was measured and calculated in percentage using the equation:

$$\% \text{ Growth inhibition} = \frac{\mathbf{NTR-TR}}{\mathbf{NTR}} X\ 100 \text{ ---------- eqn 2}$$

Where:

NTR = Inhibition diameter of untreated Plates

TR = Inhibition diameter of treated Plates.

Chemical-activity relationship

On theoretical basis, the chemical activity of the studied compounds, the quantum chemical computations of the ground state molecular geometries; polarizabilities, energies and frontier orbital energies (E_H and E_L) of 4,4'-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) and 2-(4-hydroxy-3-methoxyphenyl)propanamide were carried out using semi-empirical parameterized methods 3 (SE-PM3) molecular orbital theory in vacuum with Spartan 14. The energy optimisations of the compounds leading to the energy minima were carried out (Kosar *et al.,* 2012). Each molecule was allowed to relax, enabling all calculations to converge to the optimised geometries and correspond to an energy minimum (Kosar *et al.,* 2012). The optimised structures were then used to obtain the ground state molecular geometry parameters; dipole moment, polarizabilities, energies and the frontier molecular orbital energies of the studied molecules at the same level of theory (Anbarasan *et al.*, 2010).

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Results And Discussion

GC-MS Result

The observed principal chemical constituent in the sap is 2-(4-hydroxy-3-methoxyphenyl) Propanamide (73.05 %), and the minor was 4,4'-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) (26.95 %) (Figure 1).

UV-Vis Spectroscopy

Absorption spectra of these nanoparticles were recorded, as shown in Figure 2. The conduction band and valence bands lie very close to each other, where electrons move freely. These free electrons give rise to a surface plasma resonance absorption band, which occurs due to the collective oscillation of electrons in resonance with a light wave.

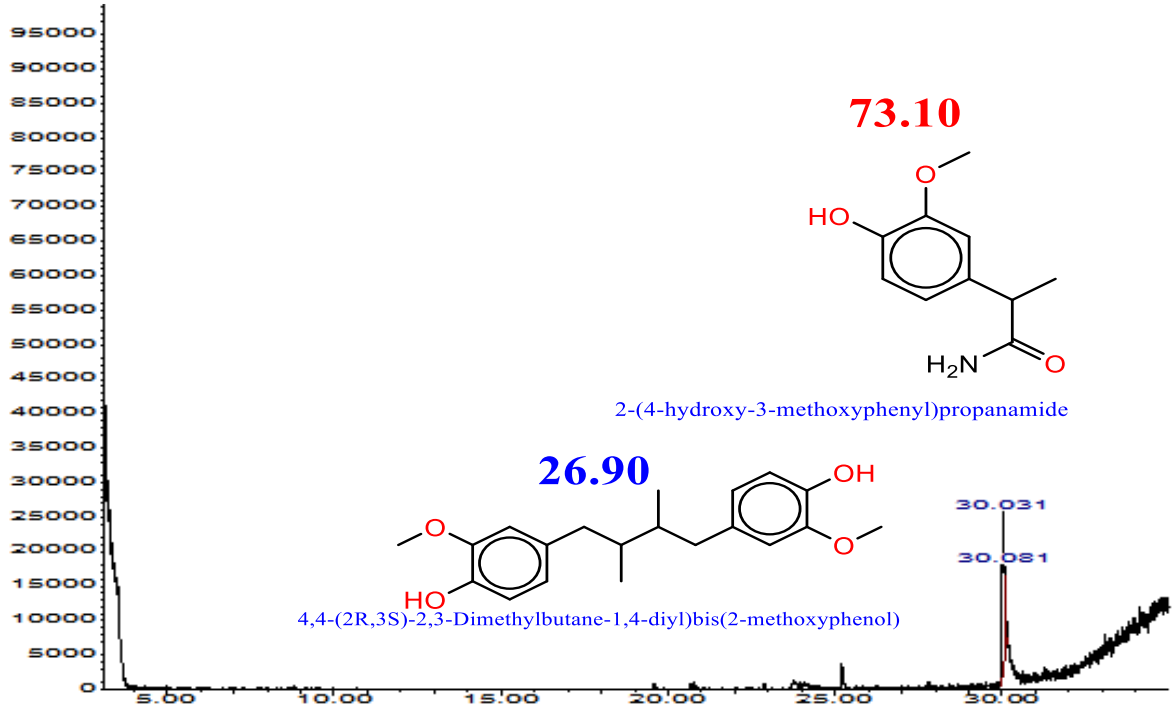

Fig 1: Gas Chromatogram of the chemical constituents of sap of *Pycnanthus angolensis*.

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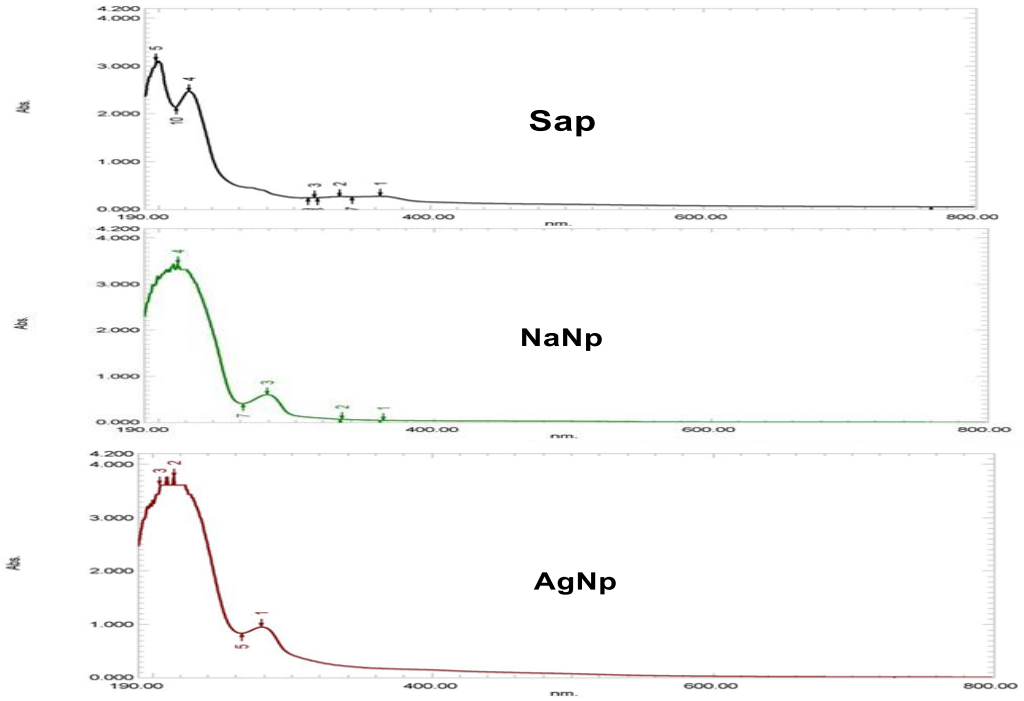

Fig 2: UV-Visible Spectra

Table 1: Absorption frequencies of the samples

Functional groups	Crude	NaNPs	AgNPs
OH	3385-3481	3452	3404-3481
C-H	2686-3184	2686-3194	2690-3184
C=N	2360	2360	2359
C=O (amide)	(1606). -1643	1612-1643	1608-1645
Terminal CH_3	1327-1421	1327-1421	1327-1421

The absorption spectra showed a Surface Plasmon Resonance, and peaks were observed between 198.5 nm and 220 nm for crude sap, between 205 nm and 278 nm for AgNPs and between 214 nm and 279 nm for NaNPs. The bathochromic shift or red shift of the AgNPs and NaNPs is an additional indication that the nanoparticles were formed. The shift to the higher wavelength means they have lower energy and hence higher activities. This was in accordance with what was reported in the literature (Edison & Sethuraman, 2013; Monalisa & Nayak, 2013).

FT-IR Result

Interpretation of FT-IR Spectra

OH/NH Functional groups

The absorption peaks at 3385 cm^{-1} and 3481 cm^{-1} of the crude sap are assignable to the NH or OH group. This suggests the presence of OH and/or NH functional groups in the two structures detected by the GC-MS chromatogram (Figure 1). For the NANPs, there was only an absorption peak at 3452 cm^{-1} representing OH. This suggests that the NH group has probably reacted with NaCl added. The peaks also appeared in AgNPs, but NH has shifted from 3385 cm^{-1} to 3404 cm^{-1}.

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Methyl, Methylene and Methine

These appeared between 2686-3184, 2686-3194, and 2690-3184 for crude, NaNPs, and AgNPs, respectively. The three functional groups are present in the two compounds (Figure 1). All the spectra showed that these groups are present in aromatic compounds because they all extend beyond 3000 cm^{-1}. It has been reported that the C-H peak absorption of aromatic compounds occurs above 3000 cm-1 while the aliphatic compounds appear below 3000 cm-1 (Alabi & Oyeku, 2017; Scott *et al.,* 2013).

Cyano compound C=N

The peak appearing at 2360 cm^{-1} for crude and NaNPs and at 2359 cm^{-1} for AgNPs indicate the presence of the cyano functional group, but this is not shown in the structures of the identified compounds (Figure 1).

Carbonyl functional group attached to amide.

It has been reported in the literature that the characteristic absorption of the amide carbonyl functional group is expected between 1680 and 1630 cm^{-1} (Akshay & Kishor, 2005; Alabi & Lajide, 2017). For crude, NaNPs and AgNPs are 1606-1643 cm^{-1}, 1612-1643 and 1327-1421, respectively, confirming the earlier reports about the functional group ($CONH_2$).

Terminal Methyl (CH_3)

The characteristic absorption band of this group is 1300 $cm^{-1.}$ For the samples at hand, the absorption peaks appeared between 1327 and 1421 cm^{-1}.

Finger print region

None of the three spectra exhibited identical features in this region. The spectra validated the compounds identified by the chromatogram (Figure 1) and further confirmed the synthesis of NaNPs and AgNPs. Although the spectra of the crude and the two synthetic compounds show some similarities, noticeable differences are still present. It is well established that no two distinct compounds can possess an identical fingerprint region.

SEM Results

Scanning Electron Microscopy (SEM) is widely employed to examine the particle morphology of samples. The results revealed that the biosynthesised NaNPS and AgNPS using *Pycnanthus angolensis* sap exhibited predominantly oval-shaped particles with smooth surfaces (Plates 2a and 2b). The only noticeable distinction was observed in the AgNPS micrograph, where some larger, yet still oval-shaped, particles appeared.

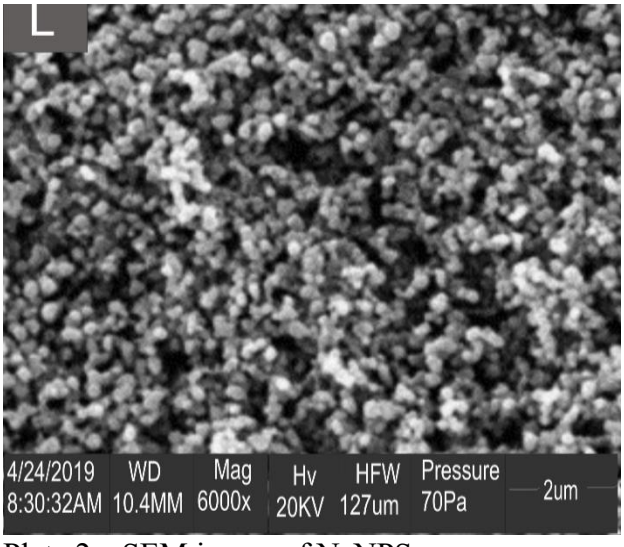

Plate 2a: SEM image of NaNPS

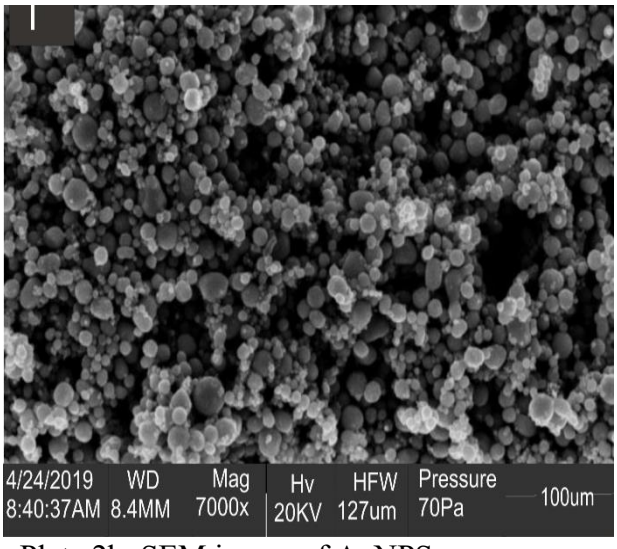

Plate 2b: SEM image of AgNPS

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Antioxidant activity

The antioxidant activities of the crude sap, AgNPS, NaNPS, and ascorbic acid (standard) were evaluated using an established standard method. As presented in Plate 3, ascorbic acid consistently exhibited the highest activity across all concentrations, followed by the crude sap and sodium nanoparticles (NaNPS) (Rios & Recio, 2005). In contrast, silver nanoparticles (AgNPS) showed the lowest activity at every concentration tested. At concentrations of 1% and 0.5%, NaNPS demonstrated more vigorous radical-scavenging activity than the crude sap; however, at lower concentrations (0.25% and 0.125%), the sap outperformed NaNPS. This indicates that the scavenging ability of NaNPS is concentration-dependent, with reduced potency at lower concentrations. The weak performance of AgNPS aligns with the antimicrobial findings, where incorporation of silver into the sap reduced its bioactivity. The crude sap, by contrast, maintained a relatively stable scavenging capacity (69%–65%). These findings further support the traditional practice of adding sodium chloride to sap for cough treatment. However, the concentration is critical—optimal efficacy is achieved when the preparation is taken undiluted (Newman & Cragg, 2016).

Antibacterial activity investigation of sap

AgNPS, NaNPS, NaCl salts, and streptomycin sulphate (standard) were screened against *Staphylococcus aureus* and *Aspergillus niger*. The antimicrobial results are presented in Table 2. The standard completely inhibited the growth of both the bacterium and the fungus, producing an inhibition zone of 5.40 mm. Sodium nanoparticles (sap mixed with NaCl) inhibited *Staphylococcus aureus* with a zone of 19 mm but showed no activity against *Aspergillus niger*. Similarly, silver nanoparticles (sap mixed with $AgNO_3$) produced an inhibition zone of 10 mm against *Staphylococcus aureus* but had no inhibitory effect on *Aspergillus niger*. (Adu-Amankwaah, *et al.* 2023).

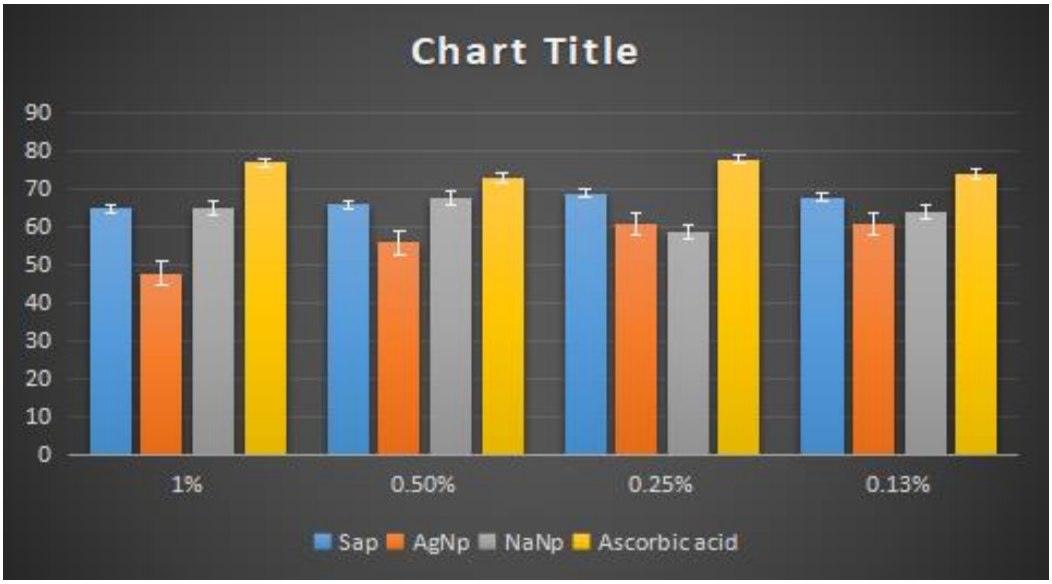

Plate 3: Radical Scavenging activities of the samples

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Table 2: Zones of Inhibition of a Bacterium and a Fungus

SAMPLE	AgNPs (mm)	NaNPs (mm)	SREP (mm)	NaCl (mm)
Staphylococcus	10	19	5.40	NI
Aspergilus niger	NI	NI	5.40	NI

STREP- streptomycin sulphate 100 micro litre (used as a control)

Theoretical Results

Plates 4 and 5 illustrate the HOMO–LUMO energy gap structures of the two compounds obtained from *Pycnanthus angolensis* sap. In propanamide, electron density is more localised in the HOMO than in the LUMO. For bis-methoxyphenol, electron density is concentrated on the left aromatic ring in the HOMO structure, whereas in the LUMO structure, it shifts to the right ring. The calculated energy gap values indicate that bis-methoxyphenol, with a slightly lower gap (8.95 eV), is more reactive than propanamide (9.05 eV). However, the difference is marginal (0.10 eV). Interestingly, this finding contrasts with the common assumption that the principal constituent dictates the chemical and biological activity of a sample (Mattys *et al.*, 2000). Theoretical analysis revealed that the minor compound, bis-methoxyphenol (26.95%), is potentially more potent than propanamide (73.05%). However, given the slight difference in energy gap, the overall potency of the sap is likely to result from the synergistic interaction between both compounds—4,4′-[(2R,3S)-2,3-dimethylbutane-1,4-diyl]bis(2-methoxyphenol) and 2-(4-hydroxy-3-methoxyphenyl)propanamide.

Distribution of Charges

Charges of 2-[4-(Hydroxy-3-MethoxyPhynyl] Propanamide

In both the HOMO and LUMO structures, electron density is highly concentrated on the benzene ring. No significant charge distribution was observed around the amide functional group, whereas partial charge delocalisation extended to the hydroxyl and ether groups, though less pronounced than on the benzene ring.

Charges of 4,4-[(2R, 3S)-2,3-DimethyButane-1,4-Diyl] Bis(2-MethoxyPhenol)

The charges are localised on the bis(2-methoxyphenol) moiety of the compound, appearing predominantly on the left side in the HOMO and shifting to the right side in the LUMO.

Conclusion and recommendations

To the best of our knowledge, this is the first study to identify the chemical constituents of *Pycnanthus angolensis* sap, as well as to synthesise and characterise its nanoparticles both experimentally and theoretically. The results revealed that the sap contains only two major constituents (Figure 1), which further validates the traditional practice of adding sodium chloride (common salt) before its use in the treatment of cough. The authors recommend that further studies be carried out on this sample.

Research implications of the study

The research implications involve investigating the scientific basis for using *Pycnanthus angolensis* sap for managing cough, including potential new treatments, pytochemical discovery, conservation and sustainability, and further research directions.

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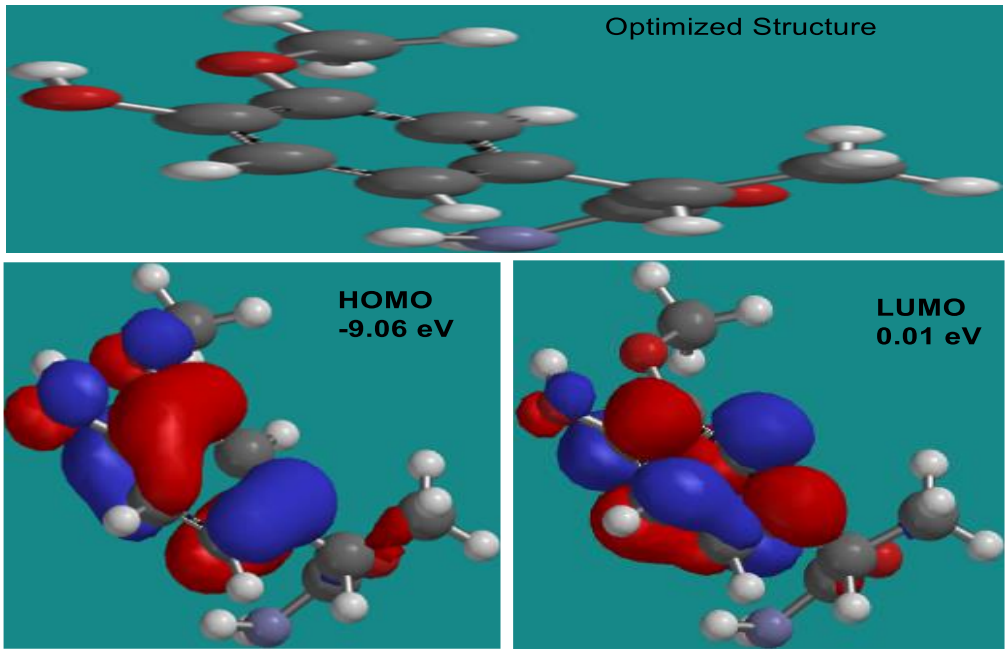

Plate 4: HOMO-LUMO Structures of 2-[4-(Hydroxy-3-MethoxyPhynyl] Propanamide

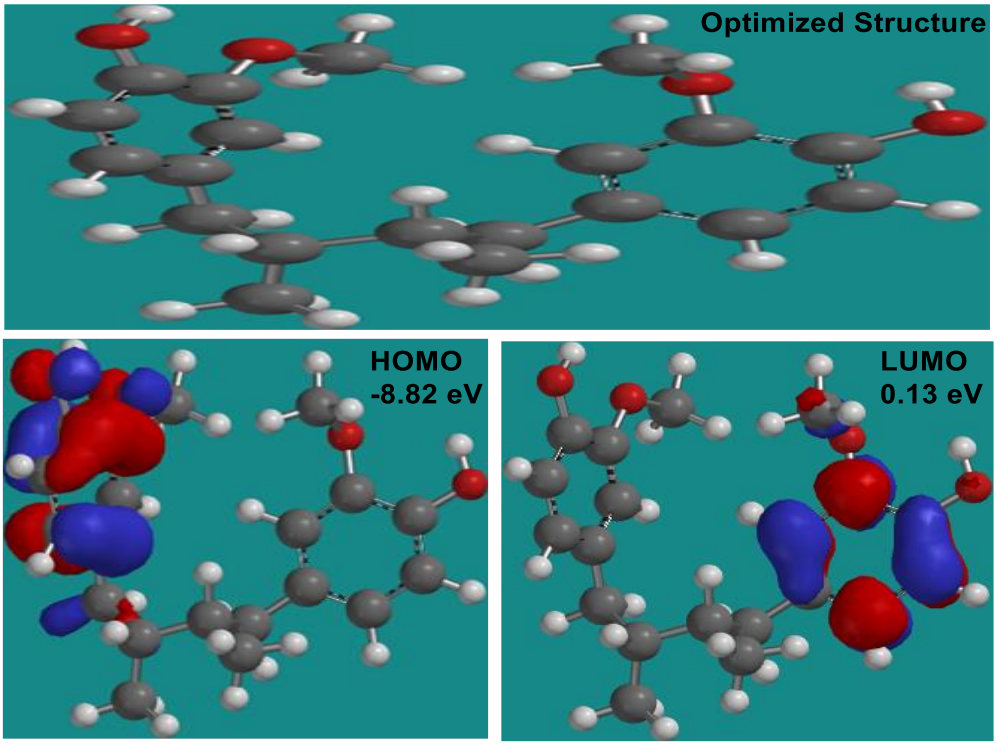

Plate 5: HOMO LUMO Structures of 4,4-[(2R, 3S) 2,3-DimethyButane-1,4-Diyl] Bis(2-Meth oxyPhenol)

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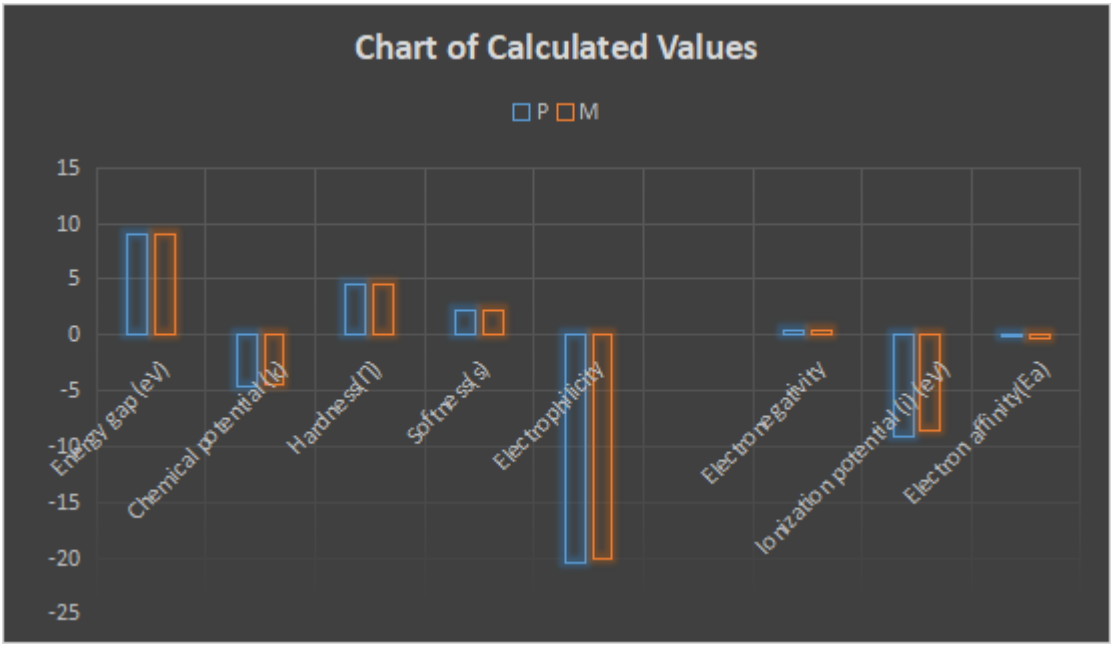

Plate 6: HOMO-LUMO Calculated values

Study limitations

The significant limitations now are the *lack of an in vivo* experiment and the limited scientific evidence. Meanwhile, traditional medicine utilises *Pycnanthus angolensis* for various ailments, including coughs. More research is therefore needed to fully understand its efficacy and safety.

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Ethical approval

In vivo has not been done, hence there is no need to seek this approval yet.

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