

*Full Length Research Paper*

# Exploring the antimicrobial modulatory potential of the sap from oil palm tree

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The sap from the oil palms (*Elaeis guineensis*), harbours complex microbiota and provides a rich source of therapeutic metabolites. This study investigated the antibacterial modulatory activity of sap from *Elaeis guineensis* on selected bacteria. To test how well the sap from the oil palm tree affects bacteria, the minimum inhibitory concentration (MIC) of some common antibiotics was found by mixing the sap from the oil palm tree with broth and measuring the results. The MIC of chloramphenicol, ampicillin, amoxicillin /clavulanic acid, and cefixime combined with the sap from the oil palm tree against the test organisms were in the range of 1.25 - 2.5, <0.0024 - 2.5, <0.0024 - 0.625, and 0.3125 - 2.5 mg/ml, respectively. There was a 4-fold and 16-fold reduction in the MIC of chloramphenicol against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, and an 8-fold reduction of the MIC of cefixime against *Staphylococcus aureus*. There was an increase in the MIC of the antibiotics in 64% of the in vitro modulatory tests. A 2 – >2083-fold increment in the MIC of the antibiotics was observed against the test pathogenic bacteria. Metabolite profiling of the sap from the oil palm tree showed the presence of simple sugars such as cellobiose, maltose, sucrose and glucose. The sap from the *E. guineensis* exerted a modulatory effect on the antibacterial activity of chloramphenicol, ampicillin, amoxicillin /clavulanic acid and cefixime.

**Key words:** *Elaeis guineensis*, antibacterial modulation, antimicrobial resistance.

## INTRODUCTION

The fresh sap from oil palm tree is very sweet and refreshing because of the presence of sucrose, but within 24 h the concentration of sucrose falls to less than 50% of the initial amounts. Microorganisms contaminate the palm sap and convert it into palm wine through a fermentation process. This fermented drink is traditionally appreciated and consumed by several people throughout the world. Fermentation virtually ends when the Ph falls

to 4.0; this whole process lasts about 48 h (Oluwole et al., 2023; Djeni et al., 2020; SatyaLakshmi et al., 2018). It is available to varied cultures worldwide as a locally manufactured drink with documented therapeutic benefits (Mbuagbaw and Noorduy, 2012). In parts of Africa, the consumption of this fermented sap forms part of many ceremonies, including weddings, funerals, and birth celebrations. This fermented sap may be served in varied

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flavours which may be unfermented (sweet), fermented (sour), or vinegary (Chung and Yousef, 2003; Eze et al., 2019). Fewer stigmas is associated with the drinking of this fermented sap in all age groups as compared to other distilled derivatives which welcomes its use in many societies in Africa by children, adolescents, and the elderly. This fermented sap is often traditionally used in some African communities to extract bioactive metabolites from medicinal herbs (leaves, stems, barks) as a treatment for a wide variety of bacterial, parasitic, and viral infections including chicken pox, measles, malaria, and yellow fever (Nwaiwu et al., 2020; Akinrotayo, 2014; Sosa et al., 2009).

The increased evolution of antimicrobial resistance in pathogenic species of microorganisms, coupled with challenges in the discovery of new antimicrobial agents necessitates the search for varied agents that may synergistically enhance the activity of antimicrobials (Ali et al., 2021; Almuhayawi, 2020; Cheesman et al., 2017; Adusei et al., 2019). Foods and nutraceuticals may be able to modify the effects of commercial antimicrobials in a way that reverses the mechanism of resistance that resistant strains of microorganisms have developed (Sibanda and Okoh, 2007). Routine consumption of the sap either alone or with meals in many societies may gradually influence the use of this sap in orally administering medicinal formulations, including antibiotics (Endo et al., 2014). Folklorically, there is a belief that taking the sap with anti-infectives increases the activity of antimicrobials against pathogenic bacteria. Some community pharmacists have observed this belief in Ghana whereby clients request antibacterial agents, intending to take them with the sap from oil palm trees. This laboratory-based study, therefore, sought to ascertain this claim and also determine whether the sap from an oil palm tree has any potential antibacterial modulating activity using selected antibiotics against common pathogenic bacteria.

## MATERIALS AND METHODS

### Sample collection

Freshly tapped sap from oil palm (*Elaeis guineensis*) was collected from a farm at Sokode Gborgame in the Volta Region, Ghana. It was directly aliquoted into sterile bottles and frozen at -20°C.

### Test microorganisms and antibiotics

The test organisms employed in determining the antimicrobial and resistance-modulating activity of the sap from oil palm were *Staphylococcus aureus* (ATCC 27853), *Proteus mirabilis* (ATCC 49565), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 33495), *Salmonella paratyphi A* (clinical isolates) and *Salmonella paratyphi B* (clinical isolates). All the typed strained organisms were obtained as frozen stock cultures from the Centre for Plant Medicine Research, Mampong, Akuapem, Ghana while the clinical strains were obtained from the Microbiology Laboratory of Central

University, Ghana. The organisms were revived by inoculating two loopfuls of the frozen culture into peptone broth (Oxoid, UK, CM0009B) and incubating at 37°C for 24 h. The cells were then suspended in Mueller Hinton broth (Oxoid, UK, CM0405B) and incubated at 37°C for 24 h. A microbial count of 0.5 McFarland standard (approximately  $1.0 \times 10^8$  CFU/mL) was used for the experiment. This was obtained by diluting the cultures with a sterile solution of 0.9% saline. The test antibiotics used in the study were pure powders of chloramphenicol, amoxicillin /clavulanic acid (co-amoxiclav), ampicillin, and cefixime obtained from Ernest Chemist Limited, Tema, Ghana.

### Preliminary investigations and LC-MS Based Metabolomics Analysis of freshly tapped sap from oil palm

Benedict's test was conducted to confirm the presence of reducing sugars (Simoni et al., 2002) while ascorbic acid and alcohol content were quantified by iodometric and back titration methods respectively (Ikewuchi and Ikewuchi, 2011). Liquid chromatography–mass spectrometry (LC–MS) was also conducted on the sap from the oil palm sample as follows. 30 ml of the sample was centrifuged at  $12,000 \times g$ . The supernatant was used for metabolomics analyses while the pellets were washed twice with sterile saline water and frozen at -80°C for later culturing and microbial diversity analyses. 1 ml of the sap from the oil palm sample supernatant was diluted in 1 ml of acetonitrile. The mixture was centrifuged at  $18,000 \times g$  for 10 min at 4°C and filtered through 0.2 µm PTFE filter into a 2 ml septum vial and used for LC-MS analysis.

### Antibacterial assay of reference antibiotics

The MICs of the reference antibiotics (chloramphenicol, co-amoxiclav, ampicillin, and cefixime) were determined against *S. aureus*, *P. mirabilis*, *S. paratyphi A*, *S. paratyphi B*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* using the broth microdilution method (Wiegand et al., 2008). The reference antibiotics at concentrations of 0.0024 mg/ml to 5 mg/mL were prepared in 96-well plates and the volumes were adjusted to 190 µL. A volume of 10 µL of test organism suspension containing  $5 \times 10^5$  CFU/mL was added to make it 200 µL per well. They were incubated at 35°C for 24 h (Andrews, 2001). The MIC was recorded as the least concentration that showed no visible bacterial growth which was detected by the absence of a purple colour after the addition of 10 µL of 0.1% w/v 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to each well followed by incubation at 37°C for 30 min.

### Determination of the antibiotic resistance modifying activities of sap from oil palm

The MICs of the reference antibiotics were determined against the study organisms in the presence of a sub-inhibitory concentration of the sap from the oil palm. The sap from oil palm (alcohol content 0.7% v/v) was used as a vehicle to dissolve the antibiotics. The required volume of the antibiotic solution was added to 100 µL of double-strength Mueller Hinton broth. The broth was adjusted to 190 µL with sterile distilled water, and 10 µL of test organism was added to produce 200 µL. The plates were then incubated at 37°C for 24 h. The MIC was recorded as the least concentration that showed no visible bacterial growth which was detected by the absence of purple colour after the addition of a few drops of 0.1% MTT to each well followed by incubation at 37°C for 30 min. The experiment was done in triplicate. Modulation factor (MF), calculated as (MIC of antibiotic alone) / (MIC of antibiotic + sap from oil palm), was used to express the antibiotic-potentiating effects of

**Table 1.** Modulatory effect of freshly tapped sap from oil palm on the antibacterial activity of selected antibiotics.

Test organisms	Chloramphenicol				Ampicillin			
	MIC of antibiotic only (mg/mL)	MIC of antibiotic + sap from oil palm (mg/mL)	MF	Δ	MIC of antibiotic only (mg/mL)	MIC of antibiotic + sap from oil palm (mg/mL)	MF	Δ
<i>S. aureus</i>	1.25	0.3125	4	4+	0.1563	1.25	0.12504	8-
<i>P. mirabilis</i>	1.25	2.5	0.5	2-	0.625	5	0.125	8-
<i>S. paratyphi A</i>	1.25	1.25	1	-	<0.0024	5	<0.00048	>2083-
<i>S. paratyphi B</i>	1.25	0.625	2	2+	0.0391	2.5	0.01564	64-
<i>P. aeruginosa</i>	2.5	0.1563	15.99	15.99+	0.0391	1.25	0.03128	32-
<i>E. coli</i>	1.25	0.625	2	2+	2.5	1.25	2	2+
<i>K. pneumonia</i>	1.25	0.625	2	2+	0.3125	5	0.0625	16-

Test organisms	Amoxicillin /clavulanic acid				Cefixime			
	MIC of antibiotic only (mg/mL)	MIC of antibiotic + sap from oil palm (mg/mL)	MF	Δ	MIC of antibiotic only (mg/mL)	MIC of antibiotic + sap from oil palm (mg/mL)	MF	Δ
<i>S. aureus</i>	0.1563	1.25	0.12504	8-	2.5	0.3125	8	8+
<i>P. mirabilis</i>	0.0098	5	0.00196	510-	0.625	2.5	0.25	4-
<i>S. paratyphi A</i>	0.0781	2.5	0.03124	32-	2.5	5	0.5	2-
<i>S. paratyphi B</i>	0.1563	5	0.03126	32-	0.3125	5	0.0625	16-
<i>P. aeruginosa</i>	0.625	2.5	0.25	4-	2.5	2.5	1	-
<i>E. coli</i>	<0.0024	2.5	<0.00096	>1042-	0.3125	2.5	0.125	8-
<i>K. pneumonia</i>	0.1563	2.5	0.06252	16-	1.25	1.25	1	-

MIC2: MIC of antibiotic + palm wine (mg/mL), (+): reduction in MIC (increased activity), (-): increase in MIC (decreased activity), Δ: Fold increase or decrease in the MIC, Amoxi/clav: amoxicillin/clavulanic acid, MF: modulatory factor.

the sap from oil palm.

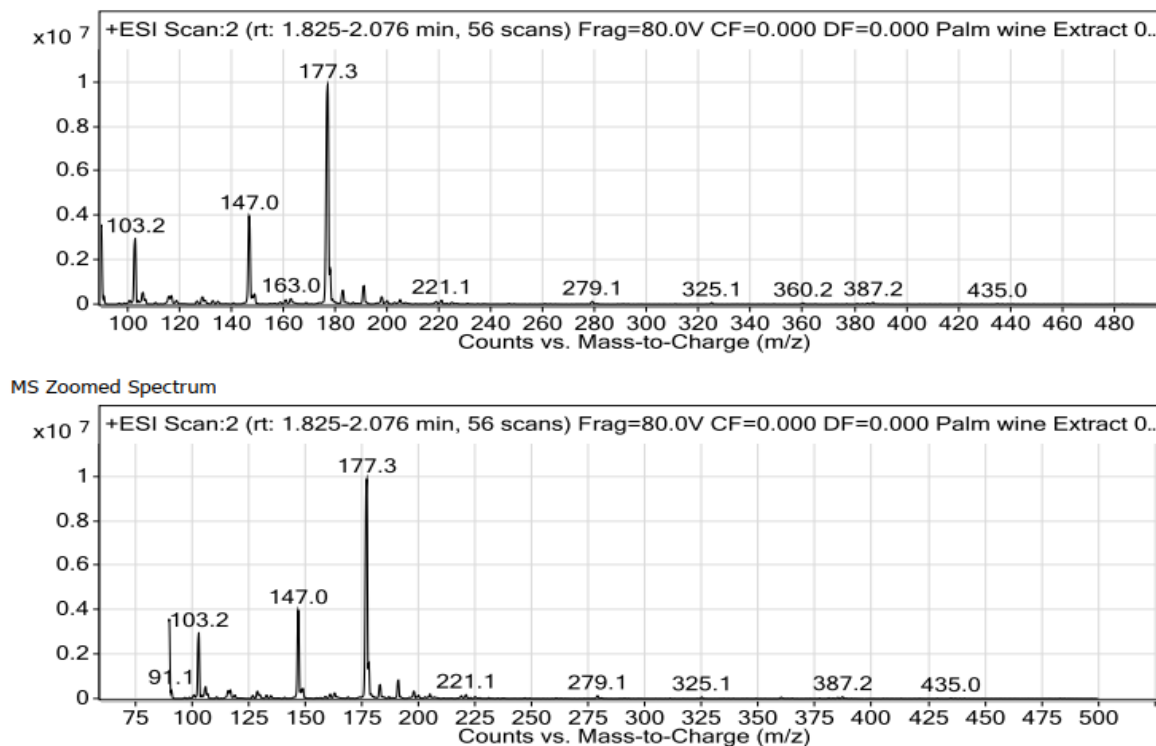
## RESULTS

### Effect of freshly tapped sap from oil palm on the antibacterial activity of chloramphenicol, ampicillin, amoxicillin /clavulanic acid, and cefixime

The MIC of chloramphenicol, ampicillin, amoxicillin /clavulanic acid, and cefixime with sap from oil

palm against all the test organisms were in the range of 1.25 – 2.5, <0.0024 – 2.5, <0.0024 – 0.625, and 0.3125 – 2.5 mg/ml respectively (Table 1). There was a resistance modifying effect of fresh sap from oil palm on the activity of chloramphenicol against all the test microorganisms except *S. paratyphi A*. A reduction in the MIC of chloramphenicol was observed against *S. aureus* (4-fold), *S. paratyphi B* (2-fold), *P. aeruginosa* (16-fold), *E. coli* (2-fold) and *K. pneumoniae* (2-fold). There was no change in the MIC of chloramphenicol against *S. paratyphi A*.

The freshly tapped sap from oil palm enhanced the activity of ampicillin through a 2-fold reduction in the MIC against *Escherichia coli*. There was an increase in the MIC of ampicillin against *S. aureus* (8-fold), *P. mirabilis* (8-fold), *S. paratyphi A* (>2083-fold), *S. paratyphi B* (64-fold), *P. aeruginosa* (32-fold) and *K. pneumoniae* (16-fold). The antibacterial activity of amoxicillin /clavulanic acid against all the test organisms was reduced (increase in MIC) in the presence of the freshly tapped sap from oil palm. The MIC of co-amoxiclav increased against *S. aureus* (8-fold),



**Figure 1.** Chromatogram from LC-MS Based Metabolomics Analysis of sap from oil palm (Compound 5, RT: 1.905).

**Table 2.** Metabolites identified from the sap from oil palm samples.

S/N	Retention time (min)	Observed m/z [Adduct(s)]	Monoisotopic mass	Molecular formula	Identification	References
1	1.487	177.2 (M+H)	176.12	C <sub>14</sub> H <sub>14</sub> O <sub>9</sub>	Ascorbic acid	Doddipalla et al. (2022) and Ezeagu et al. (2010)
2	1.487	365.2 (M+Na);	342.30	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	Cellobiose	Ezeagu et al. (2010)
3	1.487	365.2 (M+Na)	342.30	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	Maltose	Ezeagu et al. (2010)
4	1.487	381.1 (M+K)	342.30	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	Sucrose	Ezeagu et al. (2010)
5	1.905	198.1 (M+NH <sub>4</sub> )	180.16	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	Glucose	Doddipalla et al. (2022) and Ezeagu et al. (2010)

*P. mirabilis* (510-fold), *S. paratyphi* A (32-fold), *S. paratyphi* B (32-fold), *P. aeruginosa* (4-fold), *E. coli* (>1041-fold) and *K. pneumoniae* (16-fold). There was no change in the MIC of cefixime against *P. aeruginosa* and *K. pneumoniae*. A reduction in the MIC of cefixime was obtained against *S. aureus* (8-fold). However, an increase in MIC was observed in the in vitro modulatory assay of cefixime and fresh sap from oil palm against *P. mirabilis* (4-fold), *S. paratyphi* A (2-fold), *S. paratyphi* B (16-fold) and *E. coli* (8-fold).

#### Chemical constituents in sap from oil palm as determined from LC-MS Analysis

LC-MS screening of the fresh sap from oil palm

principally showed the presence of monosaccharides (like glucose), disaccharides (including cellobiose, maltose, and sucrose), and ascorbic acid (Figure 1 and Table 2). The presence of these compounds has been previously reported in the sap of oil palm tree (Doddipalla et al., 2022; Ezeagu et al., 2010). The sweet taste of the product could thus be attributed to the presence of the free sugars. Ascorbic acid, on the other hand, could be thought to contribute to the observed antimicrobial effects of the product.

#### DISCUSSION

The modulatory effect of sap from oil palm on selected antibiotics was assessed by determining the MIC of the

antibiotics with sap from oil palm against some reference organisms. A decrease in MIC of the antibacterial agent with sap from oil palm implied potentiation of the antibacterial activity by the sap from oil palm. An increase in MIC, however, suggested a decrease in the activity of the antibacterial agent against the test organism (Adusei et al., 2019).

The addition of sap from oil palm to the antibiotics did not have any resistance-modifying effect on chloramphenicol against *S. paratyphi* A. Also, there was no change in the MIC of ampicillin against *P. aeruginosa* and *K. pneumoniae*. In general, the test antibiotics' MIC (the amount they needed to kill bacteria) went down in 25% of the *in vitro* modulatory assays that were done. Considerable among these is the 4-fold and 16-fold reduction in the MIC of chloramphenicol against *S. aureus* and *P. aeruginosa* respectively, and the 8-fold reduction of the MIC of cefixime against *S. aureus*. The metabolites from fresh sap from oil palm have been reported to possess antimicrobial effects against *S. aureus*, *E. coli*, *S. typhi*, *S. dysenteriae*, and some viruses (Oluwole et al., 2023; Akinrotoye, 2014).

This finding suggests that there may be some bioactive present in sap from oil palm worth exploring that could produce a potentiating effect on the activity of some antibiotics. The modulatory effect of the fresh sap from oil palm on the selected antibiotics increased the MIC (reduction in antibacterial activity) of the antibiotics in 64% of the *in vitro* modulatory assays conducted. No modulation occurred in 10% of the tests conducted. This finding suggests that the effect of sap from oil palm on the antibacterial activity of some antibiotics is not always synergistic. Several studies conducted have established that sap from oil palm has a complex microbial diversity (Djeni et al., 2022; Astudillo-Melgar et al., 2019). Predominant among them are yeast and bacteria cells such as *S. cerevisiae*, responsible for alcohol fermentation and characteristic odour of sap from oil palm, lactic acid bacteria (*Lactobacillus* spp, known for their probiotic potential), and acetic acid bacteria (Djeni et al., 2022; Djeni et al., 2020). These are responsible for the pH reduction through the production of organic acids, which give a sour taste to the sap from oil palm, and are also, associated with the aroma, consistency, and colour of sap from oil palm by the production of polysaccharides (Santiago-Urbina and Ruíz-Terán, 2014).

Metabolite profiling of sap from oil palm by Liquid chromatography-mass spectrometry analysis has shown sap from oil palm to be a chemically rich substrate of polyphenols, vitamins, and amino acids including free sugars, organic acids, sugar alcohols, sugar acids, ketones, terpenes, and several unknown compounds (Djeni et al., 2020; Oluwole et al., 2023). These metabolites could have interfered with the activity of the antibiotics by interacting with bioactive chemically unstable structural groups causing a reduction or potentiation in antibacterial activity (Martinez-Lopez et al., 2014; Erukainure et al., 2018).

In the current study, LC-MS screening of the fresh sap from the oil palm showed the presence of ascorbic acid which may contribute to its antimicrobial activity. Ascorbic acid has been reported to exert an antibacterial effect against *Listeria monocytogenes* and *S. aureus* (Bayan, 2013). Additionally, the combination of ascorbic acid and lactic acid was also shown to be effective against *E. coli* O157:H7 (Tajkarimi and Ibrahim, 2011). The presence of other organic acids, as reported in other studies has also been shown to possess significant antimicrobial effects. Thus, the cumulative presence of these compounds in the fresh sap from oil palm could be said to contribute to the observed enhanced antimicrobial effects of the antibiotics tested.

Also, some of the metabolites could enhance the overexpression of antibiotic resistance determinants (plasmids, integrons, transposons, and antibiotic resistance genes) in the study bacteria causing a change in their susceptibility profile to the tested antibiotics (Kung et al., 2010; Bockstael and Aerschot, 2009). A study by Doddipalla and colleagues using nuclear magnetic resonance and liquid chromatography – high resolution mass spectrometry also confirmed the presence of organic acids and sugars, however, they reported that the amounts of these metabolites were significantly altered upon storage (Doddipalla et al., 2022). This, therefore, has the potential to alter any resistance modulatory activity that could be observed from the sap of oil palm tree.

## Conclusion

The findings from this research indicate that sap from oil palm obtained from *Elaeis guineensis* can modulate the antimicrobial activity of some antibiotics. This modulatory activity in some instances potentiates antimicrobial effects while in others it attenuates this effect *in vitro*. Further work will now be conducted to elucidate which bioactives in the sap from the oil palm are responsible for the modulatory effects observed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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