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# Antimicrobial Activity of *Coffea liberica* (Liberian Coffee) in Different Roasting Intensities and Varying Extract Concentrations against *Staphylococcus aureus* and *Escherichia coli*

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**Abstract.** Much prior research has shown that coffee, particularly its polyphenolic compounds like caffeine, chlorogenic acid, and melanoidins, exhibits antimicrobial properties against various pathogens. Building on this foundation, this study aimed to determine the antimicrobial activity of Coffea liberica (Liberian Coffee) with a focus on varying roasting intensities and concentrations. Specifically, it investigated the effects of different roasting intensities and extract concentrations against Staphylococcus aureus and Escherichia coli. Coffea liberica contains polyphenols such as caffeine, chlorogenic acid, and melanoidins, known for their antimicrobial properties. The coffee beans were obtained from Amadeo Artisano Coffee Farm and roasted to light and dark intensities. Aqueous extracts were prepared at concentrations of 25%, 50%, 75%, and 100% for each roast intensity using distilled water as the solvent. Cefepime served as a positive control, and distilled water as a negative control. The disc diffusion method on Mueller-Hinton agar plates was used to evaluate the extracts' antimicrobial activity. Results showed that Coffea liberica extracts exhibited no significant antimicrobial activity against Staphylococcus aureus or Escherichia coli across all roasting intensities and concentrations, with inhibition zones equivalent to the negative control. Descriptive statistical analysis confirmed no significant interaction between roasting intensity, extract concentration, and antimicrobial activity. The findings suggest the need for further research using alternative extraction methods, higher concentrations, or different bacteria to fully understand Coffea liberica's potential as a natural antimicrobial agent.

**Keywords:** Antimicrobial activity; *Coffea liberica*; *Escherichia coli*; *Staphylococcus aureus*.

## 1.0 Introduction

Coffee, one of the world's most widely consumed beverages and a staple in a typical Filipino breakfast, has gained recognition for its pleasant taste, stimulating effects, and various antimicrobial compounds (Almeda et al., 2020). Beyond its distinct flavor, coffee exhibits antimicrobial and anti-inflammatory properties attributed to bioactive substances in coffee beans, such as chlorogenic acid, polyphenols, and caffeine (Vijaya et al., 2020). Research indicates that phenolic, chlorogenic, malic, and caffeine contribute to coffee extracts' antimicrobial efficacy against gram-positive and gram-negative bacteria (Yosboonruang et al., 2022). However, most antimicrobial studies on

coffee extracts have focused on *Coffea arabica* (Arabica Coffee) or *Coffea canephora* (Robusta Coffee), highlighting the need to explore the less-studied *Coffea liberica* (Liberian Coffee).

*Coffea liberica*, a member of the Rubiaceae family, accounts for only 2% of global coffee production, making it less commercially available than Arabica and Robusta coffee (Mubarak et al., 2019). This coffee species is commonly cultivated in lowland and tropical countries such as Malaysia, Vietnam, and the Philippines (Wahab & Nor, 2018). In the Philippines, *Coffea liberica* is notably produced in the provinces of Batangas and Cavite and is popularly known as kapeng barako. This term translates to "coffee and a wild boar," reflecting its woody taste and bitter top notes, distinct from Arabica coffee's typical fruity and sweet flavors (Philippine Coffee Board Inc., 2021).

The emergence of antimicrobial resistance presents a significant threat to global public health; the World Health Organization (WHO) has acknowledged the escalating issue of antimicrobial resistance, particularly in commonly encountered pathogens (Sukri et al., 2021). Due to this, the antimicrobial properties of *Coffea liberica*, *Coffea arabica*, and *Coffea canephora* have been a subject of interest. Research indicates that liberica coffee beans exhibit the highest antioxidant activity compared to arabica and robusta coffee beans (Frisilla et al., 2022). This antioxidant activity is attributed to bioactive compounds like polyphenols, flavonoids, and tannins in liberica coffee (Maxiselly, 2023). *Coffea liberica* is known for its unique genetic makeup, distinct from arabica and canephora, which may contribute to variations in antimicrobial properties (Berti et al., 2020).

*Staphylococcus aureus*, a gram-positive bacterium, is a major human pathogen responsible for bacteremia, skin and soft tissue infections, and device-related infections (Tong et al., 2015). This bacterium is commonly found in food-producing animals and raw food, with humans being the primary reservoir (BC Centre for Disease Control, n.d.). *Escherichia coli*, a gram-negative bacterium, is part of the normal intestinal flora but can cause various diarrheal and extraintestinal diseases (Mueller & Tainter, 2023). In the Philippines, E. coli can be found in diverse sources such as irrigation water, soil, and the feces of animals like dogs, cats, and chickens (Zara & Vital, 2022).

Despite coffee's known antimicrobial properties, comprehensive studies on the antimicrobial activity of *Coffea liberica* remain scarce. Research has shown that roasting intensity significantly impacts coffee beans' polyphenol content and antimicrobial activity (Alnsour et al., 2021). Considering the limited information on the composition and antimicrobial properties of *Coffea liberica*, along with the pressing issue of multidrug resistance in *Staphylococcus aureus* and *Escherichia coli,* this study sought to elucidate the effects of different roasting intensities and extract concentrations of *Coffea liberica* on its antimicrobial efficacy against these pathogens. Understanding the antimicrobial properties of *Coffea liberica* is increasingly important, given the global demand for diverse coffee types.

## 2.0 Methodology

### 2.1 Research Design

The study employed experimental research design using a disk diffusion method to determine the effects of varying extract concentrations and roasting intensities on the antimicrobial properties of *Coffea liberica* (Liberian Coffee) extracts. For this study, two roasting intensities - light and dark and four extract concentrations - 25%, 50%, 75%, and 100% served as the independent variables. At the same time, the dependent variables measure the antimicrobial activity against *Staphylococcus aureus and Escherichia coli*. These variations produced distinct conditions relevant to the research objectives.

### 2.2 Procedure

The researchers purchased *Staphylococcus aureus* and *Escherichia coli* bacterial strains from the University of Santo Tomas—Research Center for the Natural and Applied Sciences. The *Coffea liberica* (Liberian Coffee) beans were purchased from Amadeo Artisano Coffee Farm in Amadeo, Cavite, and submitted to the Bureau of Plant Industry for authentication to ensure their identity.

After the Bureau of Plant Industry authenticated the coffee beans, they were brought to Coffee Tonya, a fresh roaster company in Poblacion, Makati City, for roasting and grinding. For its roasting conditions, since this study required two roasting intensities – light roast and dark roast, a kilo of the coffee beans were roasted at 165°C for 1 minute to produce a light roast. In contrast, another kilo of the coffee beans was roasted at 265°C for 2 minutes to

make dark-roasted beans (Rawangkan et al., 2022). After roasting the beans, both light and dark-roasted beans were ground to a third-degree or Turkish grind to make the coffee grounds as fine as possible to aid the extraction in the subsequent steps.

The ground coffee was weighed based on the percentage of the extract concentrations – 25%, 50%, 75%, and 100%. Afterward, the weighed coffee powder is then mixed with 100 mL of distilled water in different beakers to extract the appropriate amount per coffee extract concentration (Sant'Anna et al., 2016). The beakers are labeled according to their concentration and are placed on a hot plate at 150°C for 30 minutes (Norazlin et al., 2020). Then, the coffee mixtures were filtered using Whatman Filter No. 1 and were placed in Erlenmeyer flasks. Once needed for the experiment, the remaining Whatman filter paper no. 1 was punched into circles and placed under the ultraviolet light for 15 minutes to sterilize (Afroz et al., 2020). The coffee extract was poured into several Petri dishes based on their concentration and roasting intensity. Lastly, the sterilized filter papers are soaked in the coffee extract.

The materials used to prepare inoculum were vials of inactivated *Staphylococcus aureus* and *Escherichia coli* and 5 mL trypticase soy broth. The *Staphylococcus aureus* and *Escherichia coli* vials were poured into separate 5 mL tubes of trypticase soy broth. The tubes were placed inside the incubator at 37°C for 18 to 24 hours to allow the bacteria to grow. After 18 to 24 hours of incubation, the inoculum will be checked to see if the necessary turbidity was obtained using 0.5 McFarland standard (Afroz et al., 2020). If the required turbidity is reached, *Staphylococcus aureus* and *Escherichia coli* are lawned on fifteen 150 mm x 15 mm plates. Then, filter paper discs soaked with the coffee extract solution at a known concentration and roasting intensity are placed. Cefepime antibiotic discs are added as a positive control, and filter paper discs soaked in distilled water are also placed as the negative control. The agar plates are incubated at 37°C for 24 hours.

After the incubation period, the diameter of the inhibitory zone will be measured and compared (Tenover, 2019). Calipers or rulers will be used to measure the diameter of each inhibition zone after growth is deemed satisfactory. A black, non-reflecting surface is illuminated above the plate to study the zones, and the plate is subsequently lit with reflected light (Mahon & Lehman, 2019, p. 278). Furthermore, the measurement of the zone of inhibition will be the basis for determining if the bacteria is susceptible, partially susceptible, or resistant to the Liberian Coffee extract.

### 2.3 Statistical Treatment/Analysis of Data

The researchers analyzed the data collected from the experiment using descriptive statistics. The statistical test was used to describe and summarize features from the collected data quantitatively. By using descriptive statistics, the researchers can evaluate whether there will be a significant difference in the antimicrobial activity of *Coffea liberica* (Liberian Coffee) after the provided roasting intensities and variable concentrations are employed.

### 2.4 Ethical and Safety Consideration

The researchers prioritized safety, scientific, and academic integrity to ensure ethical considerations. All personal details of the farm owner and laboratory technician are kept confidential. The data are well recorded and analyzed without modification to provide reliable findings. Consequently, before starting the experimental research, the government and farm owners must obtain the necessary authorization. The farm owners and suppliers received formal and transparent information about the researchers' objectives and were free to withdraw from the study. The farm owner and suppliers are granted complete voluntary participation. This commitment to ethical principles ensures the rights and well-being of all participants while maintaining the research's integrity and validity.

## 3.0 Results and Discussion

Table 1 presents the Zone of inhibition in millimeters (mm) observed in *Staphylococcus aureus* when exposed to different light roast *Coffea liberica* (Liberian Coffee) extract concentrations of 25%, 50%, 75%, and 100%. The results are consistent across all three trials, with a measurement of 6 mm that aligns with the Zone of inhibition of the negative control, indicating no inhibition. On the contrary, the positive control group (Cefepime) exhibited a significantly wider zone of inhibition at 21 mm, categorized as susceptible based on the antimicrobial susceptibility testing standard of CLSI, confirming its effectiveness against *Staphylococcus aureus*. Based on the

data, light roast *Coffea liberica* (Liberian Coffee) extract does not possess antimicrobial activity against *Staphylococcus aureus* at tested concentrations.

The lack of antimicrobial activity in the *Coffea liberica* extract on this set of light roast trials could be attributed to the specific phytochemical composition of the extract, which could interfere with its possible antimicrobial activity. Studies on other plant extracts have shown that the presence of certain compounds like alkaloids, tannins, flavonoids, and saponins can contribute to broad-spectrum antimicrobial activities (Parnomo, 2021). For instance, caffeine in *Coffea arabica* has been reported to inhibit DNA synthesis in bacteria, while chlorogenic acid can interfere with bacterial cell metabolism (Parnomo, 2021).

**Table 1.** Zone of inhibition measured in *Staphylococcus aureus* using light roast *Coffea liberica*

| | 25% | 50% | 75% | 100% | (+) Control Group | (-) Control Group |
|---|---|---|---|---|---|---|
| **TRIAL 1** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |
| **TRIAL 2** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |
| **TRIAL 3** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |

Figure 1 shows the results of light roast *Coffea liberica* (Liberian Coffee) at 25%, 50%, 75%, and 100%, as well as the positive and negative control, against *S. aureus*.

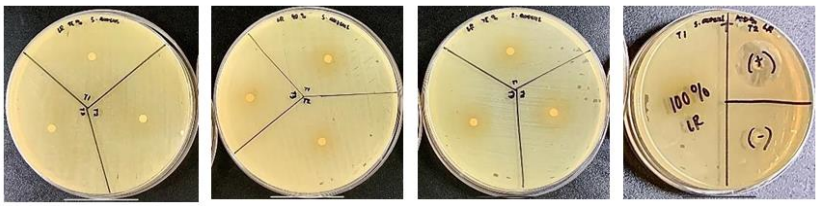


**Figure 1.** Results of light roast *Coffea liberica* against *Staphylococcus aureus*

The data presented in Table 2 indicates the Zone of inhibition measured for *Staphylococcus aureus* when exposed to various concentrations of dark roast *Coffea liberica* (Liberian Coffee) extracts. The results show that across all four concentrations, 25%, 50%, 75%, and 100%, the Zone of inhibition remained constant at 6 mm. This value is identical to the Zone of inhibition of the negative control group, which is also 6 mm, indicating no antimicrobial activity for dark roast *Coffea liberica* (Liberian Coffee) extract for these concentrations. According to Frisilla et al (2022), while it was highlighted that Liberian coffee beans have shown that they possess high antioxidant activity compared to other coffee varieties like arabica and robusta, the antioxidant potential of Liberian coffee might not directly translate into antimicrobial properties, as seen in the specific case of dark roasted *Coffea liberica* extracts against *Staphylococcus aureus*.

**Table 2.** Zone of inhibition measured in *Staphylococcus aureus* using dark roast *Coffea liberica*

| | 25% | 50% | 75% | 100% | (+) Control Group | (-) Control Group |
|---|---|---|---|---|---|---|
| **TRIAL 1** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |
| **TRIAL 2** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |
| **TRIAL 3** | 6 mm | 6 mm | 6 mm | 6 mm | 21 mm | 6 mm |

Figure 2 shows the results of dark roast *Coffea liberica* (Liberian Coffee) at 25%, 50%, 75%, and 100%, as well as the positive and negative control against *S. aureus*.

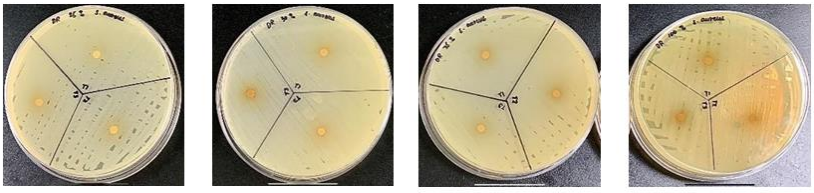

**Figure 2.** Results of dark roast *Coffea liberica* against *Staphylococcus aureus*

Table 3 shows the Zone of inhibition measured for *Escherichia coli* when exposed to light roast *Coffea liberica* (Liberian Coffee) extract at 25%, 50%, 75%, and 100%. Throughout the three trials and at the given concentrations, the Zone of inhibition remained the same with a measurement of 6 mm, similar to the negative control group, indicating that 25%, 50%, 75%, or 100% of dark roast *Coffea liberica* (Liberian Coffee) can not inhibit *Escherichia coli.* The negative result is further confirmed when compared to the Zone of inhibition of the positive control group (Cefepime) with a diameter of 25 mm, which is labeled as susceptible based on the antimicrobial susceptibility testing standard of CLSI (see Figure 3).

The antibacterial activity of coffee extracts has been attributed to compounds like caffeine, chlorogenic acid, and other plant extracts that have shown antimicrobial effects against various bacteria (Li et al., 2021). While some studies have explored the antibacterial properties of different materials and compounds against *Escherichia coli*, such as chitosan derivatives and zinc oxide composites (Wang et al., 2014), the interaction between light-roasted *Coffea liberica* and *Escherichia coli* remains understudied.

**Table 3.** Zone of inhibition measured in *Escherichia coli* using light roast *Coffea liberica*

| | 25% | 50% | 75% | 100% | (+) Control Group | (-) Control Group |
|---|---|---|---|---|---|---|
| **TRIAL 1** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |
| **TRIAL 2** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |
| **TRIAL 3** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |

Figure 3 shows the results of light roast *Coffea liberica* (Liberian Coffee) at 25%, 50%, 75%, and 100%, as well as the positive and negative control, against *E. coli*.

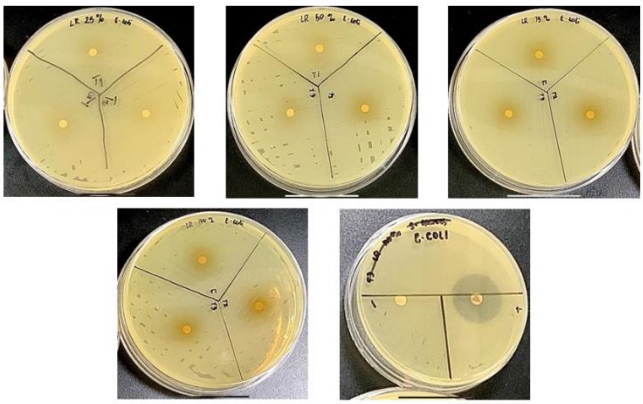


**Figure 3**. Results of light roast *Coffea liberica* against *Escherichia coli*

Lastly, Table 4 displays the Zone of inhibition measured for *Escherichia coli* when subjected to 25%, 50%, 75%, and 100% of dark roast *Coffea liberica* (Liberian Coffee) extract. The results show that the Zone of inhibition remained constant across all four concentrations at 6 mm. This value is identical to the Zone of inhibition of the negative control group, which is also 6 mm, indicating no antimicrobial activity for dark-roasted *Coffea liberica.*

**Table 4.** Zone of inhibition measured in *Escherichia coli* using dark roast *Coffea liberica*

| | 25% | 50% | 75% | 100% | (+) Control Group | (-) Control Group |
|---|---|---|---|---|---|---|
| **TRIAL 1** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |
| **TRIAL 2** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |
| **TRIAL 3** | 6 mm | 6 mm | 6 mm | 6 mm | 25 mm | 6 mm |

Investigations into the antibacterial effects of various plant extracts on Escherichia coli have revealed contrasting results. While some studies have reported susceptibility of *Escherichia coli* to certain plant extracts like Aloe vera and *Andrographis paniculata* (Adzitey et al., 2019; Anumihe, 2023), others have shown resistance of *Escherichia coli*

to extracts from plants such as *Carpolobia lutea* (Anibijuwon et al., 2018). These discrepancies underscore the complexity of plant-bacteria interactions and the need for comprehensive studies to understand the mechanisms underlying antibacterial activity. Figure 4 shows the results of dark roast *Coffea liberica* (Liberian Coffee) at 25%, 50%, 75%, and 100%, as well as the positive and negative control, against *E. coli*.

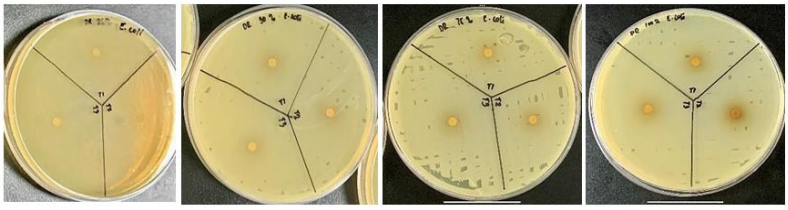

**Figure 4**. Results of dark roast *Coffea liberica* against *Escherichia coli*

This study examined the possibility of *Coffea liberica* (Liberian Coffee) aqueous extracts exhibiting antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Descriptive statistics was used to determine the effect of different roasting intensities and extract concentrations. The disc diffusion method was the principal method for assessing antimicrobial activity, with inhibition zones measured around discs containing test extract.

According to our results, neither *Staphylococcus aureus* nor *Escherichia coli* had any detectable inhibitory zones in all test instances. The experiment results show that *Coffea liberica* (Liberian Coffee) extracts lacked antimicrobial activity against these specific bacterial species at the concentrations used. Statistical analysis supported this conclusion, revealing no significant differences in antimicrobial effects between extract concentrations and the negative control group. These findings are consistent with previous research by Poire et al. (2018) and Boireau et al. (2017), highlighting the rise of multidrug resistance in *Escherichia coli*. This bacterium is known for its remarkable ability to acquire resistance genes, making it increasingly resistant to a wide range of conventional antibiotics, including penicillin, aminopenicillins, and cephalosporins. This growing resistance poses a significant challenge in treating bacterial infections and underscores the need for novel antimicrobial agents.

Despite having a lower caffeine level than other coffee species (Sithanen, 2022), *Coffea liberica* (Liberian Coffee) has been demonstrated in past studies to inhibit some microorganisms (Tan et al., 2020). Variations in the studied bacterial strains, the procedures used to prepare the coffee extracts, or the particular experimental settings could cause the observed differences between our results and those of these previous publications.

Given the acquired data, the null hypothesis was upheld, which proposed no significant interaction between roasting intensity, extract concentration, and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The results imply that *Coffea liberica*(Liberian Coffee) extracts have no antimicrobial activity against these specific bacteria under the conditions examined. Future research could investigate alternative extraction methods, higher extract concentrations, or additional bacterial species to fully assess *Coffea liberica*'s (Liberian Coffee) antimicrobial properties. Given the acquired data, the null hypothesis was upheld, which proposed no significant interaction between roasting intensity, extract concentration, and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The results imply that *Coffea liberica*(Liberian Coffee) extracts have no antimicrobial activity against these specific bacteria under the conditions examined. Future research could investigate alternative extraction methods, higher extract concentrations, or additional bacterial species to fully assess *Coffea liberica*'s (Liberian Coffee) antimicrobial properties.

**4.0 Conclusion**

The study investigated the antimicrobial effects of *Coffea liberica* (Liberian coffee) extract against *Escherichia coli* and *Staphylococcus aureus*, revealing no significant inhibitory impact on these microorganisms. Regardless of the concentration of the *Coffea liberica* extract, the results consistently showed no antimicrobial activity. Specifically, *Staphylococcus aureus* exhibited no inhibition zones when exposed to light roast *Coffea liberica* extract at concentrations of 25%, 50%, 75%, and 100%, aligning with the negative control's inhibition zones. Similarly, dark roast *Coffea liberica* extract at the same concentrations demonstrated no inhibitory effect on *Staphylococcus aureus*.

The results were analogous to *Escherichia coli*, which showed no inhibition when exposed to light or dark roast Coffea liberica extract at any tested concentrations.

The researchers concluded that both *Staphylococcus aureus* and *Escherichia coli* are not susceptible to the antimicrobial properties of *Coffea liberica* extracts, regardless of the roasting level and concentration. Despite the negative findings, this study contributes valuable knowledge by identifying the limitations of *Coffea liberica* as an antimicrobial agent against these particular bacteria. The results provide a reference point for future studies, suggesting that similar methodologies can be refined to achieve more definitive outcomes.

Future research is recommended to use fresh coffee beans, as this might alter the negative results observed in the current study. Additionally, exploring different types or sources of *Coffea liberica* or other emerging microorganisms beyond *Staphylococcus aureus* and *Escherichia coli* could offer a more comprehensive understanding of the antimicrobial potential of *Coffea liberica* extracts. Employing alternative extraction techniques and solvents, such as ethanol, methanol, or even acetone, might enhance the antimicrobial effectiveness of the *Coffea liberica*.

Addressing these concerns in future studies could unlock the potential of *Coffea liberica* as an antimicrobial agent. Extensive research encompassing a broader range of microbes, standardized procedures, and varied extraction techniques will significantly enhance our understanding. Such studies are crucial, particularly in the urgent need for sustainable antimicrobial solutions to combat the escalating issue of antimicrobial resistance.

## 5.0 Contributions of Authors

The researchers have equally contributed to the conception until the finalization of this research.

## 6.0 Funding

The researchers declare no special funding for this research.

## 7.0 Conflict of Interests

The researchers declare no conflicts of interest about the publication of this paper.

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