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Antimicrobial potential of essential oil from *Pinus caribaea* var. *hondurensis (P. caribaea)* sap

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The majority of hand sanitizers now in use include synthetic fragrances and chemical additives that pose substantial risks to human health and the environment. Ingredients like triclosan are linked to carcinogenesis, endocrine disruption, allergies, and antimicrobial resistance. This study aims to determine the constituents in pine (Pinus caribaea (P. caribaea) var. hondurensis sap and extract essential oil to be assessed for its bioactive components and antibacterial potential. Phytochemical screening of the sap was done using a qualitative method and the sap was found to contain flavonoids and alkaloids while tannins, anthraquinones and saponins were absent. Essential oil was obtained from the P. caribaea sap using the hydro-distillation process. In the GC–MS analysis that was carried out on the essential oil, it was found that the oil contained 23 bioactive compounds with the highest concentration being Sabinen (34.24%), β-Pinene (24.82%) and α-Thujene (11.5%). Other compounds such as Anethole, Linalool, Isolongifolol acetate, Camphene, Cyclopentene, γ-Terpinen, Fenchol, allo-Ocimene, Isopulegol, Levomenthol, Borneol, Citronellol, α-Longipinene and Caryophyllene were present in relatively small amounts. Assessment of the agar plates revealed that the essential oil did not show inhibitory activity to Escherichia coli (wild), E. coli (ATCC 25,922), Staphylococcus aureus (wild), S. aureus (ATCC 25,923), Pseudomonas aeruginosa (wild) and P. aeruginosa (ATCC 27,853). Whereas the essential oil did not show inhibitory activity, more studies should be carried out to evaluate the potential of essential oil from Pinus sap in the cosmetic and pharmaceutical sectors.

Keywords Antimicrobial formulation, *Pnus caribaea* sap

Between December 2019 and 26 July 2023, the global outbreak of Coronavirus (COVID-19) caused 768,560,727 million cases and 6,952,522 million fatalities.Uganda recorded 171,729 of these cases, with 3,632 deaths (WHO,[1]). COVID-19 was, just one of the many epidemics that have wreaked havoc on the planet alongside outbreaks such as Ebola, Marburg, cholera, and influenza[2,3]. The pandemic led to a change in hygiene practices with hand sanitizers becoming common household items for many individuals because they are helpful when water is not easily accessible outside or in public locations like airports, elevators, and fast-food restaurants[4]. In both public and medical settings, hand sanitizers have grown in popularity as an alternative to traditional hand washing with soap and water. Consumer usage of portable hand sanitizers and dispenser hand sanitizers was 4.1% and 14.6%, respectively, before the epidemic. These climbed following the epidemic to 39.3% for portable hand sanitizers and 89.8% for dispenser hand sanitizers[4–6].

Despite disinfectants' success in eradicating COVID-19 and other associated illnesses, concerns have been expressed regarding how their extensive use can impact animals, aquatic environments, and biodiversity. The majority of hand sanitizers now in use include synthetic fragrances and chemical additives that pose substantial risks to human, health and the environment. Ingredients like triclosan are linked to carcinogenesis, endocrine disruption, allergies, and antimicrobial resistance[7].

The lookout for natural replacements has been spurred by the rising rejection of synthetic chemicals[8]. Additionally, it's important to consider the possibility of alcohol misuse. For instance, there were several instances of exposure to sanitizers and disinfectants in Saudi Arabia, in 2020. Ingestion was the main method of exposure for both disinfectants and hand sanitizers, followed by inhalation. There were also reports of alcohol poisoning in Iran and other less developed Muslim countries with increased death and morbidity globally. Methanol poisoning was documented in every Iranian province, and some even reported deaths[9,10]. Despite the use of emollients and moisturizers in modern preparations, Alcohol based hand sanitizers (ABHSs) are connected to a range of skin reactions, including dryness, itching, irritation, pruritis, skin cracking, respiratory distress,

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swelling, redness, and a burning sensation (Ralte et al., 2021; Ugboko et al., 2020). Vomiting, conjunctivitis, coughing, and stomach ache have been shown to be the most typical side effects of both ABHSs and NABHSs. Disinfectants also change the skin's natural balance, which can lead to burns, wounds, and contact dermatitis[11].

To address the issues with most commercial sanitizers, researchers are looking for substitute components. A potential solution to the toxicity issue is the development of new hand sanitizer formulas based on botanical and natural ingredients. In place of alcohol-based hand massages, medicinal herbs with recognized antiviral and disinfectant qualities were reportedly employed during COVID-19. Herbal hand sanitizer formulations have been shown to be efficient against pathogens and also safe for human life and the environment[11].

Pinus caribaea var. *hondurensis* (*P. caribaea*) is planted throughout the tropics, including many countries of tropical Africa. This resinous tree is a source of essential oils rich in bioactive compounds such as limonene and β-phellandrene, which possess antimicrobial and insect-repellent properties[12,13].

While much research has focused on essential oils from pine stems and needles, there is limited knowledge on the phytoconstituents of its sap. Given its abundance in tropical regions, *P. caribaea* var. *hondurensis* presents a novel and untapped resource for developing natural antimicrobial formulations. This study is the first to explore the chemical composition of essential oils derived from the sap of *P. caribaea* var. *hondurensis* and assess its potential as an antibacterial agent. The findings could pave the way for innovative, plat-based hand sanitizer formulations that offer both efficacy and safety, addressing a critical need for sustainable and less harmful disinfectant solutions.

Materials and methods

Materials

Pine (*P. caribaea*) Sap was obtained from Prof Bioresearch, Uganda. Mueller Hinton agar, bacterial strains including *Escherichia coli* (*E. coli*) (ATCC 25,922), *E. coli* (wild), *Staphlococcus aureus* (*S. aureus*) (ATCC 25,923), *S. aureus* (wild), *Pseudomonas aeruginosa* (ATCC 27,853) and *P. aeruginosa* (wild) were obtained from Mbale Clinical Research Institute (Mbale, Uganda). Polysorbate 20 was purchased from Sky Chem (U) Limited (Kampala, Uganda).

Phytochemical analysis of pine sap

Pine sap was analyzed for the presence of various phytoconstituents. The qualitative determination of these phytochemical compounds was conducted according to the methods described by[14,15].

Detection of flavonoids

3 g of pine sap were immersed in 15 mL of 70% ethyl alcohol. The mixture was thereafter shaken vigorously to facilitate dissolving and filtered. 3 drops of ferric chloride solution were added to 1 mL of the essential oil.

Detection of tannins

3 g of pine sap were immersed in 15 mL of distilled water, with the intention of forming an aqueous solution. However, even after vigorous shaking, only a little part of the sap dissolved in the water. After filtration, 3 drops of ferric chloride solution were added to 1 mL of the aqueous solution.

Detection of alkaloids

3 g of pine sap were immersed in 13.2 mL of distilled water. Thereafter, 0.8 mL of sulphuric acid (with a concentration of 99%) was added to the mixture of sap and distilled water. Despite vigorous shaking, only a small portion of the sap got dissolved. Thereafter, 1 mL of wagner's reagent was added to 1 mL of the acidic solution of the extract.

Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 mL of distilled water.

Detection of anthraquinones

3 mL of the prepared aqueous extract (following the procedure for detecting tannins) was shaken with 3 mL of benzene and filtered. 5 mL of 10% ammonia solution were added to the filtrate and shaken.

Detection of saponins

3 g of pine sap were immersed in 20 mL of distilled water and then agitated for 10 min.

Extraction of essential oil from *P. carribeae* var. *hondurensis* sap

500 g of Pine sap was hydrodistilled for 3 h, using an apparatus that operated using a similar principle to the Clevenger-type apparatus[13]. The Pine sap was immersed in 10 L of water and stirred by hand to facilitate dissolving. Following the continuous heating of the mixture of water and sap at a temperature of 100 ^{0}C, a vapour mixture containing the essential oil and hydrosol moved to the condenser for conversion to liquid which was then transferred to the separator. The separator supported the separation of the essential oil from water, given that the oil is not miscible with water. The oil settled at the top of the hydrosol. The mixture was allowed to stand for 1 h and thereafter the obtained oil was collected in glass bottles and stored away from sunlight. Figure 1 shows the setup of the experiment.

Analysis of bioactive component in pine sap essential oil using Gas chromatography – mass spectrometry (GC–MS)

Analysis of the bioactive components in the oil from *P. caribaea* sap was carried out with a Shimadzu Nexis GC-2030 Gas Chromatograph and GCMS-QP202NX Gas Chromatograph Mass Spectrometer system manufactured

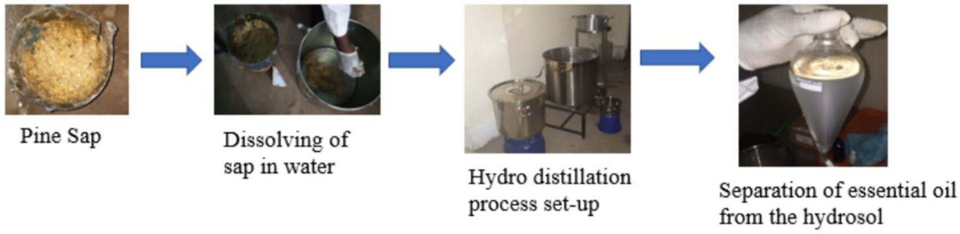

Fig. 1. Steps taken to extract essential oil from pine sap.

by Shimadzu Corporation, Japan. A Restek, Rtx-5MS capillary column (30 m, 0.25 mm, 0.25 µm) was used with helium as the carrier gas with a flow of 1.78 mL/min. The GC oven temperature was kept at 50 ^{0}C for 1 min, programmed to 140 ^{0}C at a rate of 20 ^{0}C/min, and then programmed to 300 ^{0}C at a rate of 10 ^{0}C/min. It was kept at a constant of 300 ^{0}C for 10 min. A splitless injector was used and set to 200 ^{0}C.

By matching their mass spectra with information from the NIST database, constituents were identified.

Assessment of antibacterial efficacy of essential oil from pine
The efficacy of essential oil from pine was carried out against six strains of bacteria, that is, *S. aureus* (ATCC 25,923), *S. aureus* (wild) *E. coli* (ATCC 25,922), *E. coli* (wild), *P. aeruginosa* (ATCC 27,853) and *P. aeruginosa* (wild). The test was carried out as indicated in Gilbert et al., 2014, with modifications.

Briefly, Mueller Hinton agar was prepared according to the manufacturer's instructions. The agar plate surface was inoculated by spreading a volume of the microbial inoculum (ca. 0.5 McFarland standard) over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer. The well was filled with 50 µL of the formulation at the highest concentration. Agar plates were incubated at 37 ^{0}C for 24 h. Zones of inhibition were read along two diameters. For each bacterial strain, the experiment was performed in duplicate. The antibacterial activity of *P. caribaea* oil was compared with two commonly employed oils used in herbal sanitizers, that is lemon and cedar oils.

Experimental Compliance Statement;

- The Pine Sap was collected from *Pinus caribaea* var. *hondurensis* trees cultivated by farmers in Kamuli District, Uganda (0°56′42.0"N, 33°07′30.0"E; Latitude: 0.9450, Longitude: 33.1250), as part of a commercial agreement between Prof Bioresearch (https://www.profbioresearch.net/) and the local farmers.
- Extraction of essential oils from *Carribaea* sap that was done from Prof Bioresearch, Analysis of bioactive component in Pine sap essential oils using Gas Chromatography-mass spectrometry (GC–MS) was done from the Directorate of Government Analytical Laboratory and then the qualitative assessment of the phytochemical constituents and assessment of antibacterial efficacy of essential oil from pine were done from Busitema University Natural Products Research & Innovation Centre. All these experiments were done in compliance with relevant institutional, national, and international guidelines and legislations.

Results and discussions
Phytochemical constituents of pine sap
Detection of flavonoids
A reddish colour was observed which showed the presence of flavonoids.

Detection of tannins
There was no blackish precipitate observed. Instead, A reddish colour was observed which showed the presence of flavonoids.

Detection of alkaloids
A reddish-brown precipitate was observed which showed the presence of alkaloids.

Detection of anthraquinones
There was no observable pink, red or violet colour in the ammonical (lower) phase. Instead, a milky colour was observed which showed the absence of anthraquinones.

Detection of saponins
Little Foam was observed immediately after agitation. However, it disappeared within 10 s, which showed absence of saponins.

Figure 2 shows the results from the phytochemical screening while Table 1 gives a summary of the findings.

caribaea var. *hondurensis* sap is consistent with previous research done on *Pinus* genus by[13], on the chemical composition of essential oil from *P. caribaea* needles. However, it should be noted that tannins were not observed in the essential oil from sap, and yet they were observed in *p. caribaea* needles. This could be due to the fact that oil was extracted from sap and not needles. The difference in composition could also be due to factors such as time of collection, genetic, geographic and climatic conditions[13].

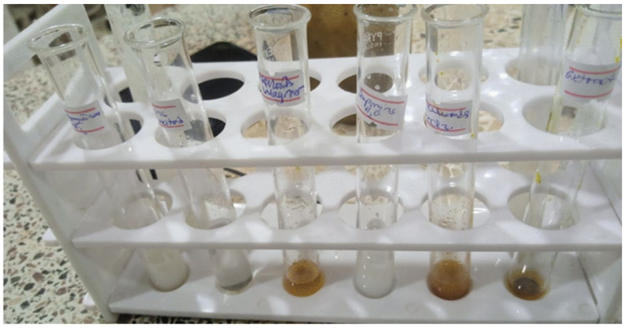

Fig. 2. Colours observed during the phytochemical screening of pine sap.

Phytochemical	Result
Flavonoids	+
Tannins	–
Alkaloids	+
Anthraquinones	–
Saponins	–

Table 1. Phytochemical screening results. '+' shows presence of phytochemical. '- 'shows absence of phytochemical. The presence of alkaloids and flavonoids from *P.*

Peak	Constituent	Synonyms for selected constituents (NIST[16])	Area (%)	Height (%)
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	α-Thujene	**3.89**	**6.73**
2	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene	Camphene	**3.44**	**9.51**
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	β-Pinene	**24.82**	**18.24**
4	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	α-Thujene	**7.61**	**8.01**
5	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Sabinen	**34.24**	**18.41**
6	Cyclopentene, 3-isopropenyl-5,5-dimethyl-		1.15	1.94
7	gamma.-Terpinene	γ-Terpinen	0.93	1.71
8	Linalool		**5.56**	**6.21**
9	Linalool		1.11	2.84
10	Fenchol		0.25	0.68
11	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	allo-Ocimene	0.63	0.78
12	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	Isopulegol	0.88	1.15
13	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	Isopulegol	0.86	1.14
14	(1R,2R,5R)-5-Methyl-2-(prop-1-en-2-yl)cyclohexanol	Levomenthol	0.27	0.74
15	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	Borneol	0.52	0.56
16	Anethole		3.56	3.88
17	Anethole		2.64	4.57
18	Anethole		2.28	4.97
19	Citronellol		0.16	0.39
20	Citronellol		0.21	0.64
21	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	α-Longipinene	0.67	1.03
22	(-)-Isolongifolol, acetate		3.95	5.05
23	Caryophyllene		0.36	0.83
		TOTAL	100	100

Table 2. Phytochemicals identified in *P.caribaea* sap. Significant values are in bold.

Bioactive compounds of pine oil

The essential oil from *P. Caribaea* sap was analysed by gas chromatography/mass spectrometry (GC/MS). A total of 23 compounds were identified as indicated in Table 2. Figure 3 shows an image of the chromatogram. Structures of the major compounds identified in the essential oil from *P. caribaea* sap are shown in Fig. 4.

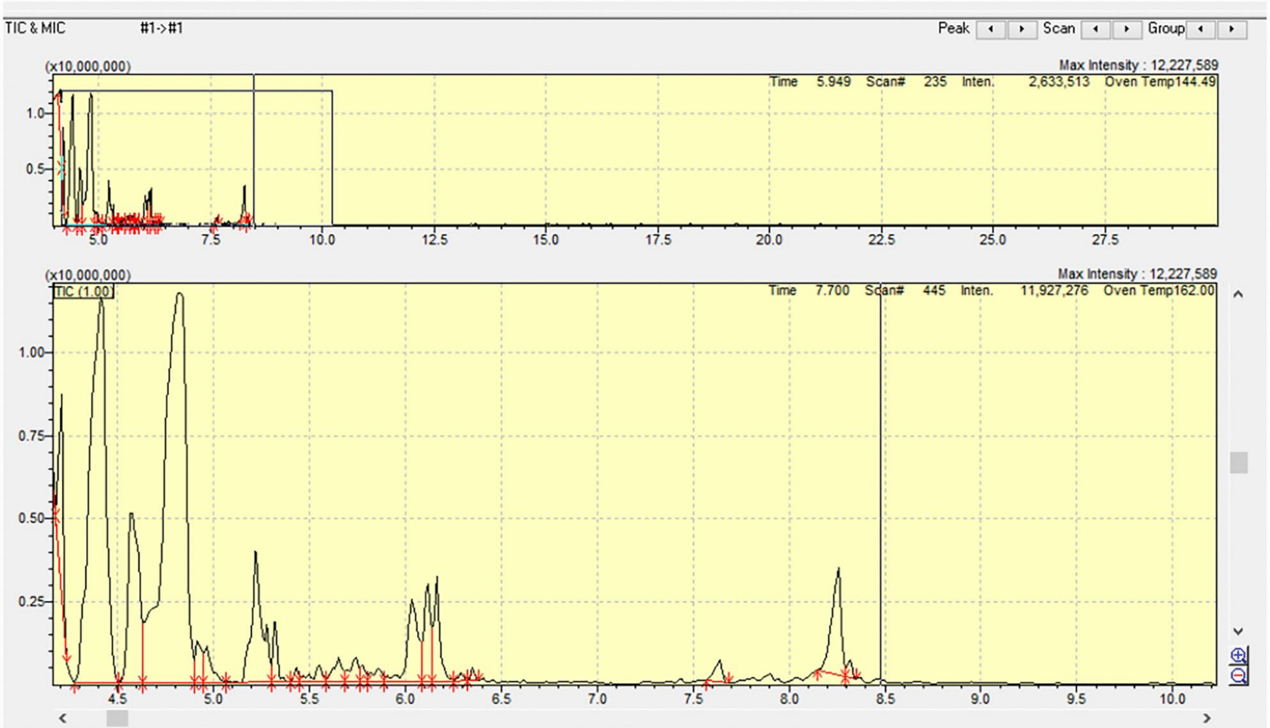

Fig. 3. Chromatogram of pine sap.

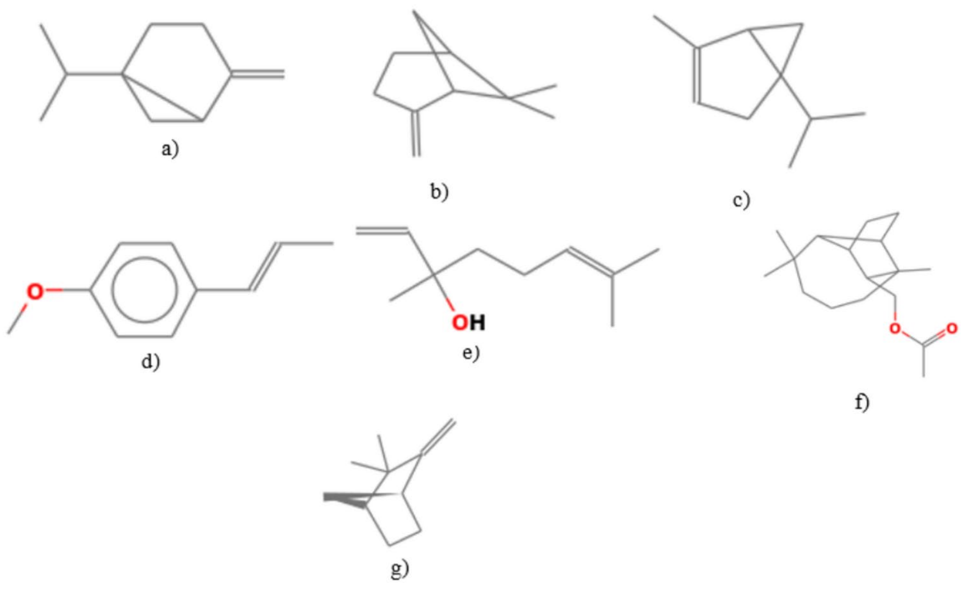

Fig. 4. Structures of the major compounds identified in essential oil from *P. caribaea* sap: a) Sabinen, b) β-Pinene, c) α-Thujene, d) Anethole, e) Linalool, f) Isolongifolol acetate and g) Camphene (NIST[16]).

The highest concentration of the constituents in the essential oil obtained from *P. caribaea* sap were Sabinen (34.24%), β-Pinene (24.82%) and α-Thujene (11.5%). Other compounds, including Anethole, Linalool, Isolongifolol acetate, Camphene, Cyclopentene, γ-Terpinen, Fenchol, allo-Ocimene, Isopulegol, Levomenthol, Borneol, Citronellol, α-Longipinene and Caryophyllene were present in relatively smaller amounts.

These findings show a unique chemical profile compared to previous studies on *P. caribaea* essential oils derived from needles, where the major components were limonene (38.6%), α-pinene (27.6%), borneol (6.7%), and myrcene (3.5%)[13]. This variation in composition could be attributed to differences in plant parts used—sap versus needles—which may affect the bioactive constituents.

Bacteria	Zone of inhibition (mm)	Standard
E. Coli (ATCC)	6.00	14.00
E. Coli (Wild)	6.00	14.00
S. aureus (ATCC)	6.00	14.00
S. aureus (Wild)	6.00	14.00
P. aeruginosa (ATCC)	6.00	14.00
P. aeruginosa (Wild)	6.00	14.00

Table 3. Inhibition activity of oil against bacteria.

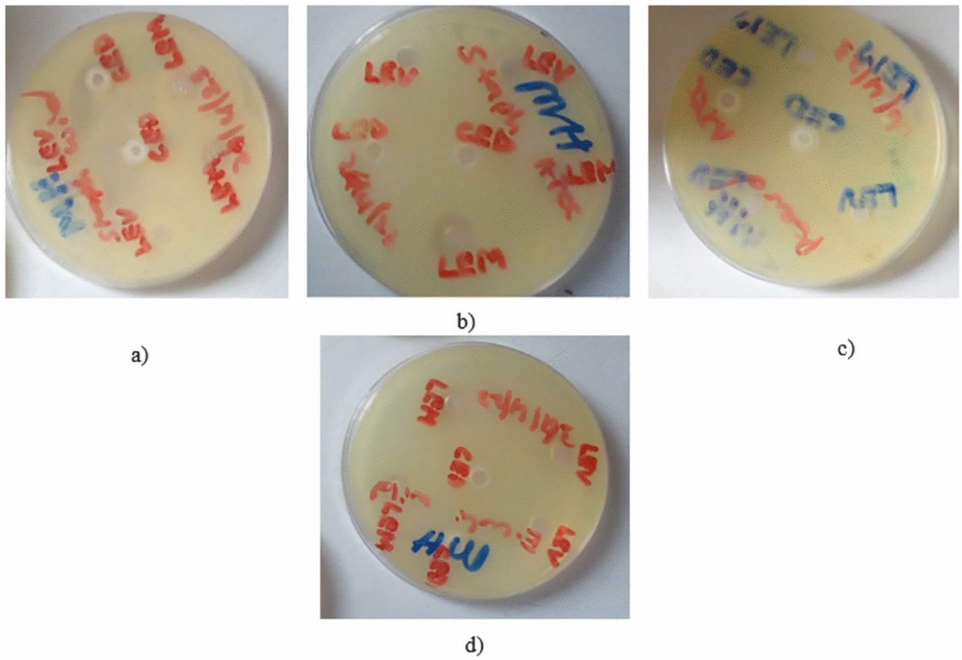

Fig. 5. Images showing Inhibitory activity of pine essential oil, in comparison with lemon oil and cedar oil, against a) *S. aureus* (wild), b) *S. aureus* (ATCC 25,923), c) *P. aeruginosa* (ATCC 27,853) and d) E. coli (Wild). LEV stands for pine oil, CED is for Cedar oil and LEM is for Lemon oil.

Biological and pharmacological potential

The essential oil from *P. caribaea* sap has great potential for use in the pharmaceutical and cosmetic industries, considering the nature of the compounds found in it. Sabinene, the dominant component, is useful for the development of therapeutic agents meant to treat infections and lessen oxidative stress. This is due to their anti-inflammatory, antimicrobial, and antioxidant properties[17].

β-Pinene, the second most abundant compound, has been studied for its antibacterial and antifungal properties, which may be beneficial in the development of natural antimicrobial formulations for wound care and personal hygiene products (Santana et al.,[18]).

Additionally, α-thujene exhibits antibacterial activity, indicating possible use in infection control. (Gözcü S and Akşit Z.[19]).

Several minor compounds further enhance the pharmacological relevance of the oil. Camphene and Borneol exhibit some degree of antimicrobial and anti-inflammatory activity indicating its potential for broader therapeutic use. (Sri et al.,[20]).

The compounds collectively enhance the versatility of *P. caribaea* sap as a source of bioactive ingredients applicable in various fields.

Antibacterial efficacy of essential oil from pine sap

The examination of in vitro antibacterial activity of the essential oil from pine showed no inhibitory effect on bacterial growth. The results of essential oil from pine sap against different bacterial species are presented in Table 3. The layout of this experiment on an agar plate is shown in Fig. 5.

In previous studies, the antimicrobial efficacy analysis of three *Pinus* species, that is *Pinus densiflora*, *Pinus thunbergii* and *Pinus roxburghaii* showed that *Pinus* species have little antimicrobial activity[21]. In this very research, it was found that *P. caribeae* did not inhibit the growth of bacillus subtills. The inhibition activity was attributed to changes in the chemical components. The results also confirmed[22]'s findings that *Pinus* species have negative activity against E. coli. However, they contradicted the author's finding that they are active against *S. aureus*. This could be still due to the differences in bioactive components as mentioned before.

Conclusions

This study contributes to the growing body of knowledge on the chemical and biological properties of *Pinus caribaea* var. *hondurensis* sap. Essential oil was successfully extracted from the sap using hydro-distillation, and phytochemical screening confirmed the presence of bioactive compounds such as flavonoids and alkaloids. GC–MS analysis revealed that sabinene, β-pinene, and α-thujene were the major constituents, highlighting the unique chemical profile of this species.

Although the antimicrobial efficacy test showed that the essential oil did not exhibit inhibitory activity against *E. coli* (wild and ATCC 25,922), *S. aureus* (wild and ATCC 25,923), and *P. aeruginosa* (wild and ATCC 27,853), these findings emphasize the need for further research. Future studies should explore the antimicrobial potential of *P. caribaea* sap at different concentrations, extraction methods, and in combination with other compounds. Such research could uncover new applications in the cosmetic and pharmaceutical industries, particularly in the development of natural products for skincare, wound healing, and infection management.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper. Should any other raw data files be needed in another format, they will be availed from the corresponding author upon reasonable request. Data will be availed from the corresponding author.

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Author contributions
M.A. Did the Conceptualization, Formal Analysis, Resources, Supervision, Data analysis, Writing-First Draft K. L. B. Performed the Conceptualization, Investigation, Methodology, Graphical, Writing First Draft, Revision K. E. Did the Conceptualization, Resources, Funding Acquisition, Project Management, Writing Revised Draft.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
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