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Original Article

Phytochemical Profile, Antioxidant and Antibacterial Activity of the Essential Oil of *Luma Chequen* (Molina) A. Gray from Peru

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ABSTRACT

Background: *Luma chequen* belongs to Myrtaceae family and it is known as "arrayan". In the traditional medicine from Peru, *L chequen* is used as aromatic plant, anti-inflammatory and hypocholesterolemic. **Objective:** To determine the phytochemical profile, evaluate the antioxidant and the antibacterial activity of *L. chequen* essential oil. **Material and Methods:** In the analysis of the volatile components a Gas Chromatography coupled to Mass Spectrometry (GC-MS) was used to identify the content of terpenes and sesquiterpenes. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was the method used to determine the antioxidant activity and obtain the half inhibitory concentration (IC_{50}). For the antibacterial activity, a colorimetric macrodilution method was carried out to evaluate the effect of the essential oil of *L. chequen* against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. **Results:** The analysis by GC-MS showed two major components alpha-pinene (62.89%) followed by 1,8-cineole (11.94%), and propanoic acid, 2-methyl-, 2-methylpropyl ester with 8.67%. In the antioxidant activity against DPPH radical, the essential oil of *L. chequen* showed an IC_{50} equivalent to124.60 ± 2.0 µg/mL. In the antibacterial activity, *L. chequen* had an MIC (minimum inhibitory concentration) for *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 of 4.35 ± 0 µg/mL and 8.71 ± 0 µg/mL respectively. **Conclusion:** *L. chequen* presented monoterpene compounds as main phytoconstituents as well as antioxidant and antibacterial activity *in vitro*. The essential oil might be used as antimicrobial agent in the future overall against *S. aureus*.

Key words: *Luma chequeen*, Antioxidant, Medicinal plant, Essential oil, Aromatic plant, Antibacterial.

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INTRODUCTION

Aromatic plants are the natural sources of essential oils (EO), which are chemical compounds of low molecular weight and volatile nature. On the other hand, essential oils generate interest in the scientific community due to the presence of promising bioactive metabolites, which have been reported antibacterial, antiviral, antifungal, insecticidal, and repellent effects, among others.[1] However, its antibiofilm capacity is the one that has generated the most interest due to resistance to multiple drugs and essential oils could be an alternative from a natural source.[2,3]

Staphylococcus aureus (a gram-positive bacteria) is one of the leading causes of bacteremia, endocarditis, cutaneous, osteoarticular, and respiratory infections.[4] On the other hand, *Escherichia coli* (a gram-negative bacteria) typically colonizes the gastrointestinal tract. Typically, *E. coli* and its human host coexist for decades in excellent health and mutual benefit. These commensal *E. coli* strains rarely cause disease unless the host is immunocompromised or in episodes like in peritonitis.[5]

Luma chequen is a perennial tree located in South American Andes between 2500 and 4000 masl. It belongs to *Myrtaceae* family, in Peru is known as "arrayan", within its medicinal uses are for gastrointestinal and respiratory disorders, migraine, and muscular pain. *L. chequen* growths in the departments of Junin, Ayacucho, Cusco, Lima, Ancash and Pasco.[6] The essential oil of leaves and twigs of *L. chequen* presented two major compounds known as α-pinene and 1,8-cineole,[7] and some biological activities have been demonstrated such as its antioxidant, antibacterial and fungicide activities.[8] Additionally, the essential oil had antimicrobial effect against *Cladosporium cladosporioides, Cryptococcus neoformans* and *Proteus vulgaris*.[9]

Currently, searching new antibacterial bioactive compounds from natural sources is still in preclinical phase but some of them are being tested in medical trials. Hence, the aim of this study was to investigate the antioxidant and antibacterial activity of the essential oil of *L. chequen* against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

MATERIAL AND METHODS

Plant material and essential oil obtention

Five kilograms of *Luma chequen* of the aerial parts were collected in the province of La Mar, department of Ayacucho, Peru (2 660 masl). The plant was authenticated in the herbarium of the Universidad Nacional Mayor de San Marcos (081-USM-2017). The aerial parts of *L. chequen* were selected and washed with a 0.1% sodium hypochlorite solution and dried until they were incorporated into a Clevenger apparatus, after two hours, the essential oil was separated by decantation and a few milligrams of anhydrous Na_2SO_4 were added to purify remaining water essential oil. Finally, the essential oil was stored in sealed amber vial at 4°C until further use.

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Gas chromatography analysis of the essential oil of *L. chequen*

The filtered sample was diluted in the proportion of 1:100 (V/V) in filtered acetone and placed in a vial. The vial was immediately positioned in the autosampler of the GC-MS system (SHIMADZU, GC-2010 Plus). 1.0 µL of the work solution was injected into the equipment in splitless mode (Split: 20:1). The sample was run on a RESTEK. RTX-5MS, 30m x 0.25 mm ID *x* 0.25 µm. The work conditions were the followings: the temperature program was 50 °C starting with increments of 3 °C/ min up to 150 °C for 10 min; and followed by increases of 3°C/min up to 250 °C for 20 min. The helium flow rate was 0.80 mL/min. Volatile chemicals were based on computer matching with the mass spectra from the NIST20 library.[10]

Antioxidant activity against DPPH radical

To carry out the antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as organic radical according to Rojas- Armas *et al*[11] with slight modifications. Our conditions to measure the antioxidant capacity were: Different conditions of the essential oil of *L. chequen* (10-500 µg/mL) diluted with dichloromethane were mixed with DPPH solution (0.01mM) prepared with methanol, 300 µL of each sample dilution reacted with 2700 µL of DPPH. Control was performed between 300 µL of methanol and 2700 µL of DPPH. Trolox at 250 mM was used as antioxidant control. After 30 minutes of reaction under dark conditions, absorbances were measured at 517 nm in an UV spectrophotometer. All procedures were done by triplicated. The formula to calculate the percentage of antioxidant activity was:

$$\text{Antioxidant activity (\%)} = [(X_0 - X_1)/X_0] \times 100$$

where X_0 is the absorbance of the control (Absorbance must be between 0.6 ± 0.05) and A_1 is the absorbance of the essential oil reacted with DPPH and corrected by the absorbance of blank. Half inhibitory concentration (IC_{50}) was determined by linear regression.

Antibacterial activity of the essential oil of *L. chequen*

To assess the antibacterial activity, a colorimetric macrodilution method was carried out to evaluate the effect of the essential oil of *L. chequen* against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Both microorganisms were kept on Trypticase Soya agar. Then each dilution was made in Müeller Hinton broth, and then was mixed with resazurin solution. The final concentrations of the essential oil of *L. chequen* ranged from 0.05 to 40 µL/mL. The positive control was ciprofloxacin following the CLSI recommendations.

Statistical analysis

Results were determined in triplicate, percentages and IC_{50} were calculated using GraphPad Prism v6 program. P values less than 0.005 is considered statistically significant.

RESULTS

Chemical profile of the essential oil of *L. chequen*

According to our results showed in Table 1, we identified 25 compounds, the analysis identified to alpha-pinene (monoterpene) as the main component of the volatile constituents with 62.89% followed by eucalyptol (11.94%), and propanoic acid, 2-methyl-, 2-methylpropyl ester with 8.67% (figure 2).

Antioxidant profile of the essential oil of *Luma chequen*

The main volatile phytochemicals were responsible of the antioxidant activity against DPPH. The essential oil of *L. chequen* exhibited a strong antioxidant activity as is shown in Table 2. Trolox also showed better antioxidant activity than essential oil with an IC_{50} of 5.4 µg/mL

Table 1: Volatile compounds of the essential oil of *L. chequen*.

Peak#	Retention Time	Name	Area%
1	3.399	1-Butanol, 2-methyl-	0.03
2	3.765	Propanoic acid, 2-methyl-, ethyl ester	1.76
3	3.951	Oxirane, tetramethyl-	0.05
4	4.075	Isobutyl acetate	0.14
5	4.160	Butanoic acid, 2-methyl-, methyl ester	0.17
6	4.535	3-Pentanone, 2,4-dimethyl-	0.70
7	4.707	Hexanal	0.06
8	6.448	Butanoic acid, 2-methyl-, ethyl ester	2.09
9	6.580	2-Hexenal, (E)-	0.24
10	6.726	Propanoic acid, 2-methyl-, propyl ester	0.85
11	7.289	Propanoic acid, 2-methylpropyl ester	0.30
12	7.750	1-Butanol, 3-methyl-, acetate	0.04
13	7.876	1-Butanol, 2-methyl-, acetate	0.68
14	10.101	Propanoic acid, 2-methyl-, 2-methylpropyl ester	**8.67**
15	11.463	alpha-Pinene	**62.89**
16	12.490	alpha-Fenchene	0.08
17	12.606	Camphene	0.73
18	15.503	beta-Pinene	2.06
19	19.880	Butanoic acid, 2-methyl-, 2-methylpropyl ester	0.55
20	21.616	Propanoic acid, 2-methyl-, 3-methylbutyl ester	0.38
21	22.187	Propanoic acid, 2-methyl-, 2-methylbutyl ester	2.16
22	23.359	Limonene	1.90
23	23.607	Eucalyptol	**11.94**
24	37.159	Linalool	1.40
25	44.875	alpha-Terpineol	0.13
TOTAL			100.00

Table 2: Antioxidant activity of *L. chequen* essential oil against DPPH.

Samples	Antioxidant activity
	DPPH,IC_{50} (µg/mL)
Essential oil of *L. chequen*	124.60 ± 2.0
Trolox	5.4 ± 0.11

Table 3: Antibacterial activity of the essential oil *L. chequen*.

Microorganism	Minimum Inhibitory Concentration (MIC)	
	Essential oil (µg/mL)	Ciprofloxacin (µg/mL)
Staphylococcus aureus ATCC 25923	4.35 ± 0	0.5 ± 0
Escherichia coli ATCC 25922	8.71 ± 0	4 ± 0

(P=0.3040) and an TEAC of 25 ± 0.01 mg ET/ g essential oil. On the other concentrations there was significant differences between EO and Trolox (P < 0.001). The equation to determine the antioxidant equivalent to Trolox is showed in the Figure 3.

DISCUSSION

Currently, the industry of essential oils extracted from aromatic plants has gained great importance due to its usefulness in various fields such as the manufacture of perfumes, cosmetic products, flavorings, in the food and pharmaceutical industry.[12] Essential oils are concentrated, aromatic and volatile hydrophobic liquids obtained from plants, they are made up of terpenoids, sesquiterpenes, alcohols, acids, acyclic esters, aldehydes and lactones.[13]

Regarding the phytochemical analysis by GC-MS of *L. chequen* essential oil, our result showed in Table 1 is similar with Vallverdú *et al,*[7] which

Figure 1: *L. chequen* plant located at La mar, Ayacucho, Peru.

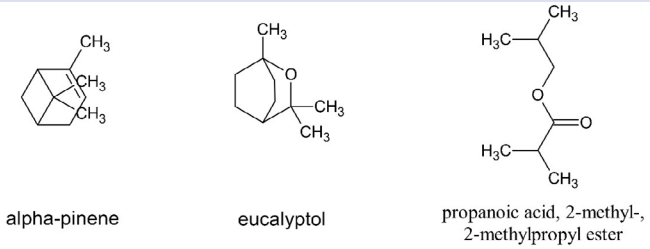

Figure 2: Main volatile components of the essential oil of *L. chequeen*.

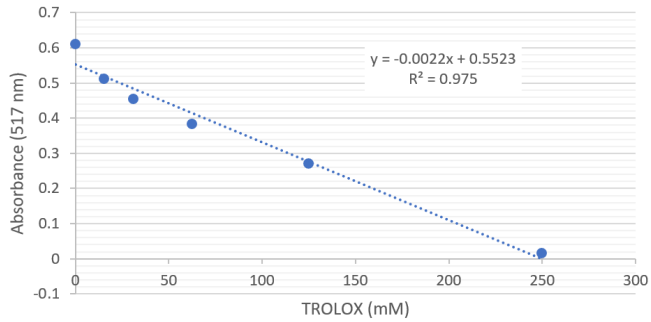

Figure 3: Regression analysis of the antioxidant activity with the standard Trolox.

showed values for alpha-pinene of 57.3% and 1,8-cineole of 7.5%, this essential oil was obtained by steam distillation at 0.72 atm and 91°C for 75 min. According to these values in some cases also depends on its extraction using conventional techniques, the temperature, soil, climate factors and other factors might be influencing in the composition of the volatile constituents.[14] In our study, the major component and representative molecule in the essential oil of *L. chequen* was alpha-pinene with 62.89% followed by eucalyptol (1,8-cineole) with 22.08%. In another study reported by Alvarado-Garcia *et al,* the essential oil of *L. chequen* from Ancash (Peru) had as main components to α-pinene (56.5%), and 1,8-cineole (8.5%).[6]

Regarding the DPPH activity, the mechanism is focused on the sample's ability to donate hydrogen to the DPPH radical, which results in the bleaching of the stable DPPH free radical from the purple color of the DPPH cation to the yellow color of diphenylpricryhydrazine. The antioxidant activity is reflected by a lower half inhibitory concentration IC_{50}. The recognized antioxidant activity of its components raised the investigation of the influence of this plant in diseases characterized due to its etiology and complications related to oxidative stress such as diabetes, dyslipidemia and atherosclerosis.[15]

On the other hand, the main microorganisms related to urinary tract infections are enterobacteria, especially *Escherichia coli*, considered the most prevalent etiological agent, responsible for 80% of infections. Due to the problems associated with the treatment of various infections, especially antibiotic resistance, herbal substances have acquired new perspectives, such as the growing interest in their use in the search for antimicrobial compounds. According to the World Organization Health around 65 to 80% of the population does not have access to primary health care and resort to traditional medicine in search of relief for many diseases. Previous studies have shown that *L. chequen* produces active metabolites that have antibacterial properties that are capable of destroying or stopping the growth or multiplication of bacteria.[7]

In a study for both Gram-negative and Gram-positive bacteria, 1,8-cineole altered the morphology and size of bacterial cells. In addition, these bacteria treated with this monoterpene compound induced apoptosis (*S. aureus*) because they exhibited a strong condensation of nuclear chromatin in the central nucleoplasm and necrosis (*E. coli*) because there was a clear reduction of nucleoplasm and nuclear chromatin accumulated in the nuclear membrane. Furthermore, 1,8-cineole is more effective against *E. coli* than *S. aureus* because, unlike S. *aureus*, the cell walls and membranes of *E. coli* cells were already compromised.[16]

CONCLUSION

We concluded that essential oil of *L. chequen* obtained by steam distillation had as main component to alpha-pinene and the antioxidant activity against DPPH radical showed a good inhibitory capacity similar to Trolox standard. Furthermore, it had better antibacterial effect against *S. aureus* ATCC 25923 than *E. coli* ATCC 25922 in the colorimetric method.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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