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Bioactivity of Ilex paraguariensis Extracts: Antimicrobial and Antioxidant Effects Against Clinical Isolates

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Abstract

Background: Natural bioactive substances with antibacterial and antioxidant qualities are abundant in medicinal plants. The biological activities of *Ilex paraguariensis* (yerba mate) can be related to its phytochemical composition that contains polyphenols and flavonoids. **Objective:** The present study aimed to analyze the antibacterial and antioxidant properties of aqueous and methanolic extracts from Ilex paraguariensis against several clinical microorganisms. **Methods:** Clinical samples of both Gram-staining positive and negative bacterial strains and yeast were collected and identified by the standard laboratory methods. Well diffusion agar test was employed to evaluate the spectrum of antimicrobial activity at different doses (60, 120, 240, and 480 mg/mL). The DPPH test was used to assess the free radical scavenging activity. **Results:** In comparison to the aqueous extract, both extracts demonstrated antibacterial activity against the studied microorganisms with the aqueous extract demonstrating significantly larger zones of inhibition than the methanolic extract. The aqueous extract was relatively greater activity against (-ve Gram) bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae* meanwhile the methanolic extract was more selective against Candida albicans. The difference in activity could be due to the difference in solubility and extraction efficacy of bioactive compounds. The aqueous extract was found to have higher antioxidant activity than the methanolic extract due to higher number of water-soluble phytochemicals present in the aqueous extract. **Conclusion:** According to the findings, Ilex paraguariensis has intriguing antibacterial and antioxidant properties, particularly its aqueous extract. It might be a natural source of bioactive chemicals with therapeutic potential; nevertheless, more in vivo and phytochemical investigations are needed to determine its efficacy and mechanism of action.

Keywords

Ilex paraguariensis; antimicrobial activity; antioxidant activity; aqueous extract; methanolic extract; clinical isolates

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1. Introduction

For a long time, medicinal plants served as a major source of bioactive substances and remain an important direction for the research and development of new antimicrobial agents to this day [1]. This paper focuses on Yerba mate (*Ilex paraguariensis*), an herbal infusion widely consumed in South America [2]. This plant is rich in active substances including polyphenols flavonoids and xanthines and existing studies have confirmed that its crude extracts and isolated monomers can inhibit a variety of Gram-staining positive and negative bacterial strains and yeast [3].

Yerba mate contains a variety of bioactive components that support its pharmacological and nutritional value: caffeine and theobromine, which belong to the methylxanthine alkaloid class, can stimulate the central nervous system; chlorogenic acid and caffeic acid, categorized as polyphenols, have strong antioxidant activity; quercetin and rutin, members of the flavonoid class, exert anti-inflammatory and antibacterial effects [4,5]. Furthermore, mate saponin, a triterpenoid saponin component of yerba mate exerts anti-inflammatory and cholesterol-lowering effects [6]. Yerba mate also contains tannins, B-complex, C, E, and D vitamins, along with Fe, Mg, and K; all these components collectively underpin its diverse biological activities and health benefits [7,8].

Accordingly, the present study aimed to evaluate the antibacterial potential of aqueous and methanolic extracts of *Ilex paraguariensis* against selected microorganisms that were isolated in laboratory conditions using diffusion-based inhibition zone measurements.

2. Materials and Methods

2.1 Microorganisms and Identification

The isolates included in this study were collected from postgraduate students at the Department of Biology-College of Science / Mustansiriya University. All isolates were documented in the table below identified using the VITEK system and then subcultured on suitable media to ensure proper activation before analysis.

The documented isolates included (with frequencies were reported in Table 1): *Acinetobacter baumannii (2), Klebsiella oxytoca (2), Klebsiella pneumoniae (4), Klebsiella aerogenes (1), Staphylococcus aureus (4), Staphylococcus epidermidis (2), Escherichia coli (3) and Candida albicans*.

Table 1: Clinical microbial isolates used in the study

Microbial Isolates	Frequency	Gram Stain	Type of specimen
Acinetobacter baumannii	2	Gram (-)	Burn wound swab
Escherichia coli	3	Gram (-)	Wound
Klebsiella pneumoniae	4	Gram (-)	Urine
Klebsiella oxytoca	2	Gram (-)	Urine
Klebsiella aerogenes	1	Gram (-)	Urine
Staphylococcus aureus	4	Gram (+)	Wound
Staphylococcus epidermidis	2	Gram (+)	Wound
Candida albicans		Yeast	Oral infection

2.2 Preparation of Aqueous Extract

Dried leaves (500 g) of Yerba mate were soaked in 1000 mL water [9]. The extract was filtered and dried in an incubator for 3 days at 50°C. A stock solution was prepared and working concentrations were prepared by serial dilution to obtain four reported concentrations: 480, 240, 120, and 60 mg/mL.

2.3 Preparation of Methanolic Extract

A Soxhlet apparatus containing 400 mL of methanol was used to extract 100 g of dried leaves over the course of four hours. The resulting extract was dried in an incubator for 2 days at 50°C [10]. A stock solution was prepared and serially diluted to obtain the same four reported concentrations: 480, 240, 120, and 60 mg/mL.

2.4 Well Diffusion Agar Test

MHA agar was used for the well diffusion agar method of antimicrobial screening [11]. Microorganisms were grown in nutrient broth for 24 hours. Agar wells (8 mm diameter) were prepared in each Petri dish and loaded with the four concentrations of extract (480, 240, 120, and 60 mg/mL). Following a full day of incubation at 37°C with distilled water serving as a negative control, inhibition zones were measured as the total diameter including the well (8 mm). At lower extract concentrations, antimicrobial activity was minimal, resulting in inhibition zones only slightly larger than the well diameter (e.g., 9–10 mm) in some replicates, while other replicates showed no measurable inhibition. This led to considerable variability among triplicates and consequently higher standard deviation values.

2.5 Antibiotic Susceptibility Testing

Antibiotic sensitivity testing was also documented using the diffusion disk method on (MHA) agar following CLSI guidelines [12]. Inhibition zones were measured to the nearest millimeter and interpreted. Table 2 detailed antibiotic susceptibility table was included for multiple isolates and

antibiotics (e.g., vancomycin, gentamicin, amikacin, trimethoprim/sulfamethoxazole, ofloxacin, cefoxitin) with R/I/S interpretations.

Table 2: Antimicrobial Susceptibility Results According to CLSI Guidelines (2024–2025)

Bacteria	Vancomycin	gentamicin	amikacin	Trimethoprim/ sulfamethoxazole	Ofloxacin	cefoxitin
Acinetobacter baumannii 1	R	R	R	I	R	-
Acinetobacter baumannii 2	R	R	R	S	-	I
Escherichia coli 1	R	R	R	R	-	R
Escherichia coli 2	R	R	R	R	-	R
Escherichia col 3	R	R	R	R	-	R
Klebsiella pneumoniae 1	R	R	R	R	R	-
Klebsiella pneumoniae 2	R	R	R	R	R	-
Klebsiella pneumoniae 3	R	R	R	R	R	-
Klebsiella pneumoniae .4	R	R	R	R	R	-
Klebsiella oxytoca .1	R	R	R	I	I	-
Klebsiella oxytoca .2	R	R	R	S	I	-
Klebsiella aerogenes	R	R	R	R	R	-
Staphylococcus aureus 1	S	S	S	S	-	S
Staphylococcus aureus 2	R	I	R	R	-	R
Staphylococcus aureus 3	R	R	R	R	I	-
Staphylococcus aureus 4	R	R	R	R	-	R
Staphylococcus epidermidis 1	I	R	R	I	-	R
Staphylococcus epidermidis 2	R	R	R	I	-	R

(R)Resistant / (I)Intermediate / (S)Sensitive

2.6 Antioxidant Activity

By [13] we test the radical scavenging activity of both plant extracts by adding 1 mL of each extract to 1 mL of freshly prepared DPPH solution. After a 30-minute reaction carried out in the dark absorbance is recorded at the 517 nm wavelength using an ultraviolet-visible spectrophotometer.

Radical scavenging capacity (%) = (A blank) – (A extract) / (A blank) x 100

Where: A (blank)= the absorbance of DPPH with methanol

A (extract)= the absorbance of DPPH mixed with plant extracts

2.7 Statistical Reporting

Data was analyzed using ANOVA at $P < 0.05$ [14]. Results are presented as mean inhibition zone (mm) ± SD where reported. LSD (0.05) values were provided for extract activity tables. We were making three replicates for all experiments.

3. Results and Discussion

3.1. Antimicrobial activity of *Ilex paraguariensis* extracts

The clinical isolates of Gram-staining negative bacteria (*A. baumannii, E. coli, Klebsiella*), Gram-staining positive bacteria (*S. aureus and S. epidermidis*) and yeast (*Candida albicans*) by the agar well diffusion method at four concentrations (60–480 mg/mL). This agrees with the previous studies which have reported the bioactivity of plant extracts against bacteria (*E. coli* and *S. aureus*) and confirms the overall antimicrobial activity of Ilex species observed, according to the composition of the extracts, and the assay conditions [15,16].

A positive correlation was seen with the concentration for the aqueous extract, and this was seen to be consistent across the board [17]. This dose–response pattern supports the biological plausibility of the observed effect and indicates that the concentration of active constituents is adequate to inhibit microbial growth in diffusion conditions [18]. One of the remarkable findings of the present work is that the aqueous extract has more potent effect in most of the bacterial targets as compared to the methanolic extract (Tables 3 and 4). Similar attention has been drawn to water extracts in the literature [19,20], and the fact that the water extract is often chosen for antimicrobial screening because of its predicted content and activity such as hydroxycinnamic acid derivatives and other polar compounds has been reported [20]. It should be noted that inhibition zones approaching the well diameter must be interpreted with caution, as small absolute differences in measurements may result in relatively large standard deviations.

Table 3. Antimicrobial activity (inhibition zones) of the aqueous extract of *Ilex paraguariensis*

Microorganisms	The average of inhibition zone in (mm) (mean ± SD) (n = 3)				Mean
	480 mg/ml	240 mg/ml	120 mg/ml	60 mg/ml	
A. baumannii	**24.33 ± 0.57**	**22 ± 1**	**13.66 ± 1.15**	**9.33 ± 0.57**	**17.33**
E. coli	**36.33 ± 1.52**	**31.33 ± 1.15**	**21 ± 6.55**	**18.66 ± 1.15**	**26.83**
K. pneumoniae	**35.66 ± 2.08**	**25.66 ± 2.08**	**24.33 ± 1.52**	**16 ± 3.6**	**25.42**
S. aureus	**29.66 ± 0.57**	**26.66 ± 1.52**	**23 ± 1**	**20.66 ± 0.57**	**25.00**
S. epidermidis	**27.66 ± 2.51**	**26 ± 4**	**21.33 ± 0.57**	**20.33 ± 2.51**	**23.83**
Candida albicans	**28.66 ± 1.15**	**27.66 ± 1.52**	**20.66 ± 0.57**	**17.66 ± 0.57**	**23.67**
L.S.D (0.05) = 1.78					

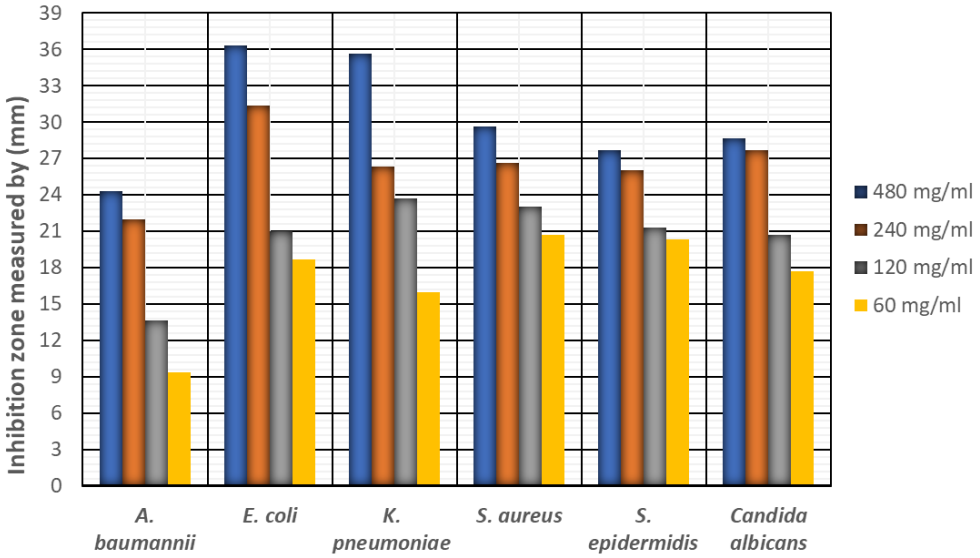


Figure (1): The inhibitory effect of aqueous extract on different microbial species.

3.2. Differences in susceptibility to microorganisms (aqueous extract dominance)

The *E. coli* and *K. pneumoniae* (Gram-staining negative bacteria) were highly responsive to the aqueous extract as evidenced by the large inhibition zone as reported in Table 3 with the maximum concentration used. Plant polyphenols and their analogues can be intrinsically less permeable in Gram-negative bacteria, but they can also act by several mechanisms such as disrupting membranes, binding to proteins, and causing oxidative stress [21]. These polyphenols as components of yerba mate phytochemistry (polyphenols, flavonoids, caffeine, tannins) interact with cell wall/membrane proteins [22]. Importantly, earlier work [23,24] has also been reported on the bioactivity of aqueous extracts against *E. coli* and *S. aureus*, which corroborates the credibility of the antibacterial signal observed here.

When tested with the aqueous extract, the same results were obtained for Gram-positive isolates (*S aureus and S epidermidis*), where clear inhibition was achieved at all concentrations, showing that the activity is not limited to a particular taxonomic group [25]. Since Gram-positive bacteria with a dense peptidoglycan layer and the outer membrane are missing, they could be more directly exposed to the phenolic compounds diffusing through the agar [26]. This general pattern of broad inhibition with water extracts has been reported in antimicrobial screening studies of Ilex water extracts [27].

3.3. Methanolic extract: lower antibacterial activity with selective antifungal effect

The methanolic extract had comparatively less antibacterial activity compared to most of the microorganisms tested while some of the microorganisms exhibited either less or no inhibition at intermediate and low levels of the extract (Table 4). The reason of this difference as you attribute it to the fact that under the extraction conditions you used, the secondary metabolite of yerba mate are more water soluble than methanolic, is plausible, given your study design and should be reported as an extraction-yield/composition issue, not as a definitive absence of antibacterial compounds in methanolic fractions [28].

Interestingly, the methanolic extract showed relatively greater activity against Candida albicans compared to the tested bacterial isolates (Table 4), indicating selectivity in antifungal activity for the methanolic extract. This selectivity may be attributable to differential extraction, either because the compounds have higher binding affinity for fungal membranes or higher binding affinity for fungal targets [29]. Previously, antimicrobial studies with the extracts from Ilex have been performed, where fungal pathogens, such as Candida albicans, were also included, which further validates the importance of testing fungal pathogens along with bacteria when characterizing Ilex bioactivity [30]. These results corresponded with earlier studies [31,32] reporting that bioactivity of yerba mate against both Gram-staining positive and negative bacterial strains.

Table 4. Antimicrobial activity (inhibition zones) of the methanolic extract of *Ilex paraguariensis*

Microorganisms	The average of inhibition zone in (mm) (mean ± SD) (n = 3)				**Mean**
	480 mg/ml	240 mg/ml	120 mg/ml	60 mg/ml	
A. baumannii	**13.33 ± 2.3**	**6.66 ± 0.77**	**6.66 ± 0.77**	**0 ± 0**	**6.67**
E. coli	**11.33 ± 0.7**	**3.66 ± 6.35**	**0 ± 0**	**0 ± 0**	**3.75**
K. pneumoniae	**11.33 ± 1.2**	**13.33 ± 1.52**	**0 ± 0**	**0 ± 0**	**6.17**
S. aureus	**19.33 ± 2.08**	**13.66 ± 2.51**	**2.33 ± 0.57**	**3.66 ± 6.3**	**14.25**
S. epidermidis	**11.66 ± 0.57**	**3.66 ± 6.35**	**0 ± 0**	**0 ± 0**	**3.83**
Candida albicans	**23.33 ± 1.52**	**18.33 ± 1.52**	**17.66 ± 2.08**	**15.33 ± 2.1**	**18.67**
L.S.D (0.05) = 2.30					

Note: Inhibition zones were measured including the 8 mm well diameter. High standard deviation values at low concentrations reflect variability between replicates with minimal or no inhibition, where some measurements were close to the well diameter (8 mm).

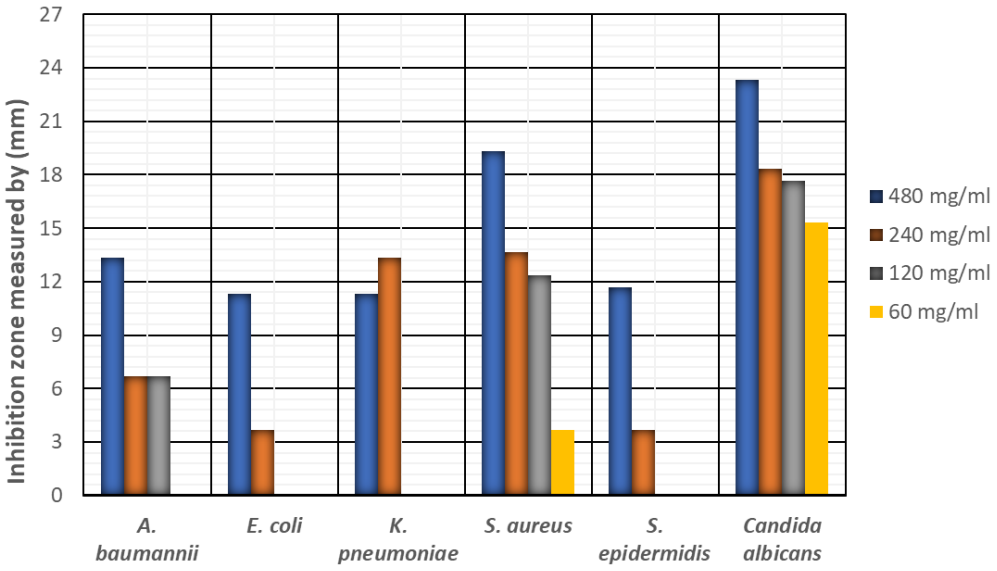


Figure (2): The inhibitory effect of methanolic extract on different microorganisms' species.

3.4. Antioxidant activity (DPPH)

To assess the antioxidant efficacy of each extract (aqueous and methanolic), the radical scavenging test by DPPH was employed as the method is widely used and it is a quick and reliable method of determining antioxidant activity [33]. The results indicated the presence of scavenging activity in all extracts with electron or hydrogen donation mechanism [34]. Of these, the aqueous extract showed the highest antioxidant activity (71.47%), meanwhile, the methanolic extract (10.26%) as shown in Table (5) and Figure (3). These results agree with earlier studies [35,36] reporting antioxidant activity of *Ilex paraguariensis* against various microorganisms.

Table 5: Scavenging radical activity of each extract of Yerba mate

	Absorbance (A)	Radical scavenging capacity (%)
Control	**0.312**	**0**
Aqueous extract	**0.089**	**71.47%**
Methanolic extract	**0.28**	**10.26%**

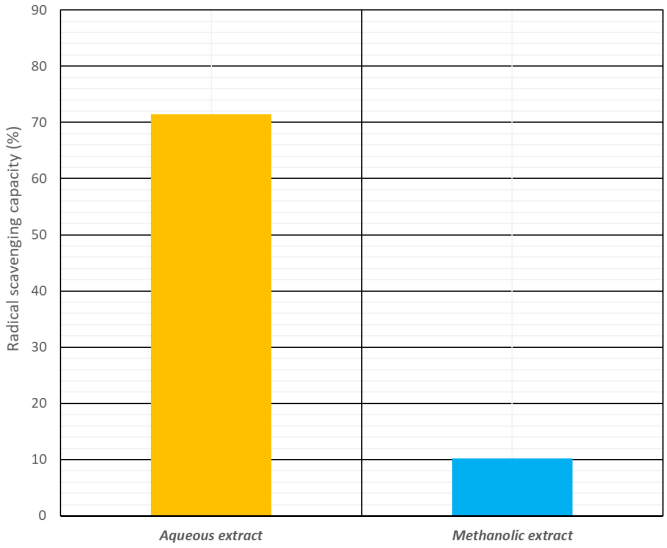


Figure. 3: Distribution rates of Radical scavenging capacity (%) for each Yerba mate extracts

5. Conclusion

The present study shows that extracts of *Ilex paraguariensis* have significant antimicrobial and antioxidant activity against important clinical pathogens. The aqueous extract exhibited better antibacterial activity, indicating that water-soluble bioactive compounds play a major role, the methanolic extract exhibited selective activity in particular against Candida albicans. Both extracts showed an inhibitory effect against free radicals, and this was more pronounced in the aqueous extract. Despite these promising findings, limitations such as in vitro conditions and the limited number of isolates necessitate further investigation. In general, *Ilex paraguariensis* is a natural source that has the potential to be developed as therapeutic agents for combating antimicrobial pathogens and antioxidants.

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