Original Article

Phytochemical Screening, Antibacterial Activity, and Chromatographic Study of *Camellia sinensis*

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Abstract

Background: This study highlights the use of plants as a vital part of Iraq's health care. One of the most significant plants for medicine is *Camellia sinensis*. **Objectives:** phytochemically and chromatographically evaluation of *C. sinensis* and its antibacterial activity. **Materials and Methods:** The phytochemical analysis revealed the occurrence of alkaloids, tannins, flavonoids, and phenols, but glycosides are not found in extracts of *C. sinensis* leaves. Antibacterial efficacy of the herbal extracts revealed that both aqueous and ethanol extracts showed some degree of inhibition of growth in *Salmonella* spp., *Escherichia coli, Streptococcus pyogenes, Streptococcus pneumonia, and Staphylococcus aureus*. **Results:** Nine functional groups were identified through Fourier-transform infrared spectrophotometer, and 10 phytochemical compounds were identified through gas chromatography–mass spectrum in the ethanol extract. The antibacterial activity of aqueous and ethanolic green tea extracts was tested. Both types of extracts showed clear biological activity against the studied bacterial species; the inhibitory effect increased with increasing concentration, with the highest activity of ethanolic and aqueous extracts against *Salmonella* spp.(inhibition zone 40, 36 mm, respectively) at 200 mg/mL, but less inhibitory effect appears on *S. pyogenes* when using aqueous extract (12 mm) at 50 mg/mL and (18 mm) at 200 mg/mL. **Conclusions:** Various therapeutically active compounds are present in the aqueous and ethanolic extract of *C. sinensis,* which encourages its use in the treatment of microbial infections. Further investigations are needed for the chemical composition of green tea, and other techniques like HPLC may be used. Further tests are necessary for its antimicrobial activity, either in vitro or in vivo.

Keywords: Antibacterial activity, aqueous extract, *Camellia sinensis*, ethanol extract, FT-IR, GC–MS

Introduction

Tea (*Camellia sinensis*) is a drink that is enjoyed by people across the world, particularly in Asian countries like China, Korea, and Japan.[1,2] Ancient civilizations have a long history of medicine.[3] many decades ago, plants were used as an origin for different medicinal drugs and are still the starting point for many modern medications. Despite the fact that plant medicine gave rise to the newer drug industry, synthetic methods of drug advancement have now become the standard. Because of their enormous capacity for biosynthetic production, plants remain an important source of medicinal drugs because of their enormous capacity for biosynthetic production.[4,5] In response to the failure of alternate drug development techniques, there has recently been a resurgence of interest in natural compounds.[6] The polyphenols of green tea, totally well-known as catechins, make up 30%–40% of the extractable solids of leaves from dry green tea. Those involve epicatechin, gallate, epigallocatechin, and epigallocatechin gallate (EGCG), each of which shows bactericidal action towards a variety of bacteria, including Gram-positive and negative.[7]

Green tea (*C. sinensis*) contains significant amounts of catechins and the polyphenols derived from them. Polyphenols are pharmaceuticals and therapeutic

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advantages, including anti-inflammatory, anticarcinogenic, antimicrobial, antidiabetic, and antioxidant capabilities.[8]

Green tea has antibacterial properties that can combat numerous bacterial pathogens that can cause skin infections, cystitis, dental caries, and diarrhea.[9] Catechins exhibited the largest antibacterial efficacy versus (G+ & G-) bacteria like *E. faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium,* and *Escherichia coli*. EGCG demonstrated potent bactericidal action toward *S. aureus* producing penicillinase and methicillin-resistant *S. aureus*.[10]

The crude extracts of catechins also prevented the vegetative growth of *Bacillus cereus*, *Clostridium botulinum*,[11] and *Enterococcus faecalis*.[12]

Catechins possess numerous antimicrobial strategies. Epigallocatechin gallate destroyed the bacterial cell membrane, fatty acid production inhibition, and repression of enzyme functioning. It is demonstrated that catechins prevent bacterial DNA gyrase from functioning, which results in bacterial cells dying. The other potential mechanism is that it may influence the action of the dihydrofolate reductase enzyme that is required by the bacterial pathogen to make purine and pyrimidine, in addition to enhancing the epidermis thicknesses.[13]

The research was conducted to achieve the following aims: Identification of the phytochemical profile of extracts obtained from *C. sinensis* by phytochemical analysis, chromatographically using a Fourier-transform infrared spectrophotometer (FTIR), and through gas chromatography–mass spectrometry (GC–MS) to evaluate the extracts' antimicrobial capability toward a selection of bacterial pathogens.

Materials and Methods

Preparation of extracts

Aqueous plant extract

A conical flask containing 10 g of the plant's powder that dried in the air was used, added 250 mL of distilled water and the mixture was boiled for 2 h, then filtered with muslin cloth eight layers of muslin cloth; after that, centrifuged at 5000 RPM for 10 min. Collected the filterate[14] and concentrated it in the oven at 45°C until it was wholly dry. The dry extract was preserved at 4°C in order to be used later.[15]

Alcoholic plant extract

About 10 g of the powdered plant was added to 200 mL of ethanol alcohol (70%) in a flask of 250 mL, covered the flask by a cotton plug, and shaken at about 140–220 RPM for 24 h. Filtered this mixture with a muslin cloth (eight layers) and put in a centrifuge at 5000 RPM for about 10 min. Then collected the supernatants. Concentrate the extract at the oven at 45°C until it was completely dry. The dry extract was preserved at 4°C in order to be used later.[15]

Phytochemicals qualitative tests

Test of alkaloids

The reagent of tannic acid Mix 2 mL solution of 10% tannic acid with 5 mL extract. Alkaloids give a precipitate of orange color.[16]

Test of phenols Test for lead acetate: dissolve 50 mg of extract in about 5 mL of distilled water, then combine with 3 mL of lead acetate (10%). The formation of a bulky white precipitate suggested the phenol compound's occurrence.[17]

Test of tannins Ferric chloride: A mounts of plant extract was mixed with water and filtrated; then 1% of ferric chloride ($FeCl_3$) was added. The emergence of a blue-green color indicates the presence of tannins.[18]

Test of flavonoids Mix 10 g of the dried plant in approximately 50 mL of 95% ethanol solution, later nominate the mixture via A. 10 mL of 50% ethanol solution, and mix with about 10 mL of potassium hydroxide (50%) and nominated B. An equal part of solutions A and B should be combined; the emergence of yellow is a sign of flavonoids.[19]

Test of glycosides Fehling's test, which is used to detect glycosides occurrence by combining equal amounts of Fehling's A solution (copper sulfate and distilled water) with Fehling's B solution (sodium hydroxide and potassium tartrate within DW), After adding a few drops from Fehling's reagent to about 5 mL of plant extract and boiling it, if sugars are found, red precipitate forms.[17]

FTIR

We processed a powder of *C. sinensis* leaves by adopting FTIR spectroscopy (Japan, Shimadzu, IR Affinity). Samples were activated in the IR region 400 and 4000 nm.[20]

The determinate of phytochemicals by GC–MS

The plant extract was analyzed by GC–MS controlled by a computer at 70 eV.[21,22] About 1 µl of the ethanolic extract was injected into the GC–MS, and the skimming was completed within 45 min. When the contents were separated, they moved into a detector that could produce an electronic signal anywhere a constituent could be identified. These signals obtained were processed by the computer. Then obtained signal had been analyzing with a computer. The injection time to elect was used to determine the retention time.

Antibacterial activity test

Stock solution (200 mg/mL) was prepared for aqueous extract and ethanolic extract depending on the method given in the study of Al-Sa'ady.[23] The serial dilutions were arranged from stock solution by sterile distilled water to get three dilutions (200, 100, 50) mg/mL. By

using the agar-well diffusion method, evaluate the antibacterial activity against three species of bacteria, including *Salmonella* spp., *E. coli*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, and *S. aureus.* Negative control was done by using sterile distilled water. After 1 h, prediffusion time was allowable at room temperature. Incubated for 18 h at 37°C. The inhibition zone was measured (in millimeters), duplicates were taken, and the mean of them was taken.

Ethical approval

The study protocol was reviewed and approved by a local ethics committee, according to document number 5234, on November 24, 2022, to get this approval.

Results

Qualitative tests of phytochemicals

It is clear from Table 1 that there are different types of phytochemicals in *C. scienensis* leaves using the aqueous and the alcoholic extract. Qualitative analysis of these extracts showed the presence of phenolic compounds, alkaloids, and tannin, while glycosides were not found in both aqueous and alcoholic extracts.

FTIR

The data in Table 2 represent the spectrum of FTIR that is used to recognize the functional groups of the active compounds, depending on the peak value of the infrared region. Figure 1 shows the peak value and active compounds present in the alcoholic extract of tea leaves, the sample run at region 400 and 4000 nm. It has been shown that there are a number of active groups in this extract. Dominant peaks were observed at 921.97 cm^{-1} represents Alkenes, peaks at 1018.41–1377.17 cm^{-1} represent alkyl halides, peaks at 1413.82 cm^{-1} represents aromatic, peaks at 1610.56 cm^{-1} represent amide, peaks at 2854.65–2922.16 cm^{-1} represent alkane.

Chromatographic separation (GC–MS) of the *C. sinensis*

The results in Table 3 represent the profile of chromatogram analysis of ethanol extract of *C. sinensis* leaves; 10 compounds have been found in this plant. These compounds shown in Figure 2, these compounds were variable and had many chemical natures, including propanoic acid, ethyl ester, cyclohexasiloxane, dodecamethyl, tetradecamethylcycloheptasiloxane, 1,3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl, neophytadiene, caffeine, (Z)-1,3-phytadiene, eicosane, pentadecanoic acid, 14-methyl, and *n*-hexadecanoic acid.

Antibacterial activity test

It was observed through this study that green tea extracts of two types (aqueous and ethanolic) showed clear biological activity against the studied bacterial species, whether Gram-positive or Gram-negative [Table 4]. This activity increases with increasing the concentration of the extract used; the higher the concentration, the higher the biological efficacy of it.

The more obvious biological activity of the ethanolic extract than that of the aqueous extract [Figures 3 and 4]. The studied bacterial species vary in their sensitivity to the green tea extracts tested in this study. In this study, the inhibitory effect of green tea varied according to the type of bacteria, with the highest activity of ethanolic and aqueous extracts against *Salmonella* spp. and *E. coli*, both of which were Gram-negative bacteria. In contrast, those

Table 1: Qualitative tests of phytochemicals

Phytochemicals		Alkaloids	Phenols	Tannins	Flavonoids	Glycosides
C. sinensis	Aqueous	+	+	+	+	-
	Alcoholic	+	+	+	+	-

Table 2: FTIR peak values of solid analysis of *C. sinensis* leaves

No.	Peak (wave number cm^{-1})	Inten-sity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area	Type of intensity	Bond	Type of vibration	Functional group assignment	Group frequency
1	921.97	80.755	0.353	925.83	891.11	3.028	0.029	Strong	=C–H	Bending	Alkenes	650-1000
2	1018.41	65.504	11.041	1085.92	927.76	23.417	5.506	Strong	C–F	Stretch	Alkyl halides	10001400
3	1242.16	84.097	2.514	1290.38	1209.37	5.59	0.546	Strong	C–F	Stretch	Alkyl halides	10001400
4	1315.45	85.759	1.119	1346.31	1305.81	2.555	0.089	Strong	C–F	Stretch	Alkyl halides	10001400
5	1377.17	84.83	0.459	1381.03	1348.24	2.185	0.05	Strong	C–F	Stretch	Alkyl halides	10001400
6	1413.82	83.609	1.112	1429.25	1390.68	2.888	0.126	Medium	C=C	Stretch	Aromatic	14001600
7	1610.56	83.285	0.261	1612.49	1562.34	3.321	0.044	Bending	N–H	Stretch	Amide	1550-164
8	2854.65	88.593	3.783	2870.08	2812.21	2.026	0.291	Strong	C–H	Stretch	Alkane	2850-3000
9	2922.16	84.451	6.126	2951.09	2881.65	3.79	0.862	Strong	C-H	Stretch	Alkane	2850-3000

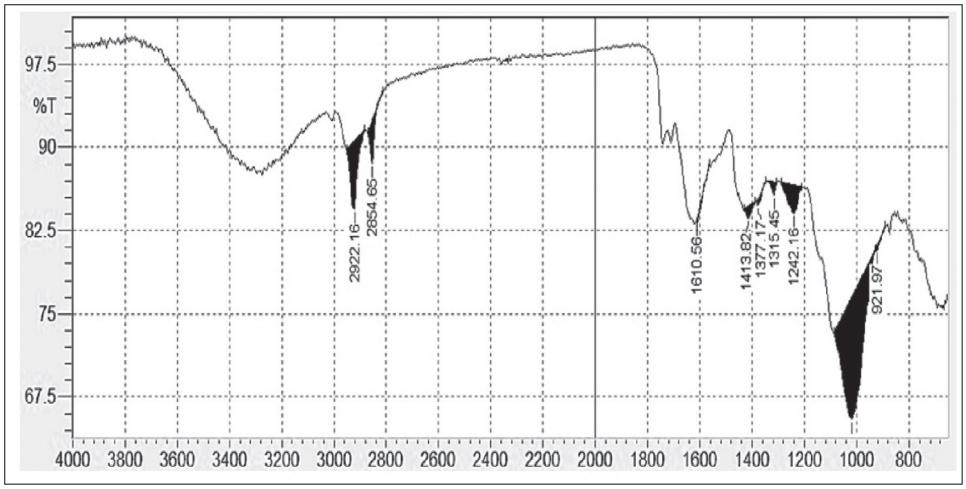

Figure 1: Fourier-transform infrared spectroscopic profile solid analysis of *C. sinensis*

Table 3: Compounds detected by GC–MS in the ethanolic extract of the leaves of *C. sinensi*

Peak no.	R.Time (min)	Name of compound	Area	% Composition by area	Chemical formula	Molecular weight	Structure of compound
1	5.073	Propanoic acid, ethyl ester	52680	0.23	$C_5H_{10}O_2$	102.1317	
2	19.276	Cyclohexasiloxane, dodecamethyl	1619008	6.94	$C_{12}H_{36}O_6Si_6$	444.9236	
3	23.694	Tetradecamethylcycloheptasiloxane	1576078	6.76	$C_{14}H_{42}O_7Si_7$	519.07	
4	32.061	1,3-Buten-2-one, 4-(4-hydroxy-3-methoxyphenyl	1108814	4.75	$C_{11}H_{12}O_3$	192.21	
5	32.375	Neophytadiene	4274729	18.33	$C_{20}H_{38}$	278.5157	
6	32.781	Caffeine	7004360	30.03	$C_8H_{10}N_4O_2$	194.19	
7	33.267	(Z)-1,3-Phytadiene	1201647	5.15	C20H38	278.5	
8	33.667	Eicosane	587019	2.52	$C_{20}H_{42}$	282.5475	
9	34.153	Pentadecanoic acid, 14-methyl	2958088	12.68	$C_{17}H_{34}O_2$	270.4507	
10	34.844	*n*-Hexadecanoic acid	2941999	12.61	$C_{16}H_{32}O_2$	256.4241	

extracts subsequently showed less inhibitory effect against Gram-positive bacteria, which included *S. pyogenes*, *S. pneumoniae*, and *S. aureus*.

Discussion

The results of the phytochemical investigation demonstrated the presence of alkaloids, flavonoids, and tannins and a lack of terpenes, saponins, and glycosides in methanolic extract, but in aqueous extract showed the presence of flavonoids, saponins, tannins, alkaloid and absence of terpenes, glycosides. This result was agreed with Lateef[24] and Lee *et al*.[25] who show that the constituents like tannins, reducing sugars, and proteins found in the extracts from green tea could be the cause of antioxidant activity. Most therapeutic plants are efficient due to they consist of active substances.[26] These secondary plant metabolites have known biological functions. Alkaloids have been shown to have effects against diabetes, arrhythmia, hypertension,

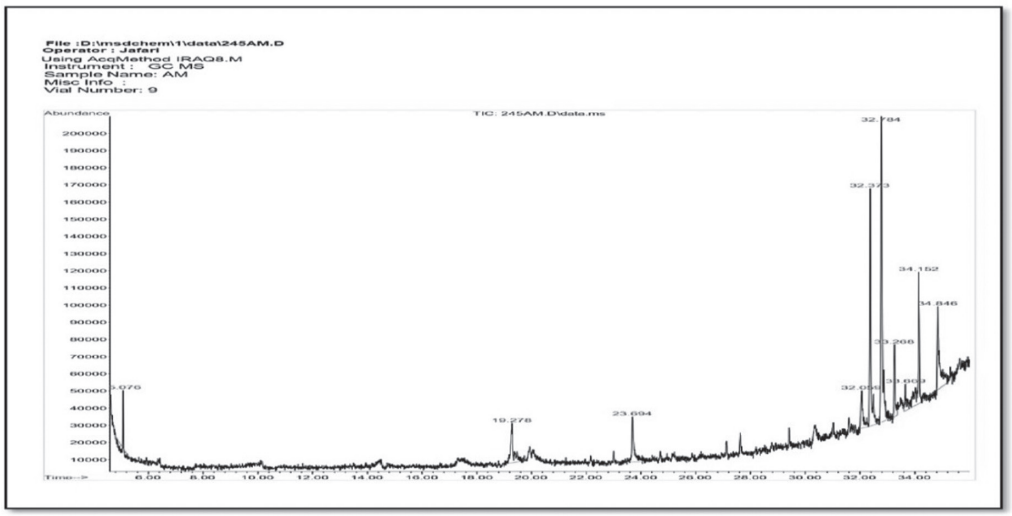

Figure 2: Gas chromatography–mass spectrum (GC–MS) analysis

Table 4: Diameters of inhibition zones of green tea *(Camellia sinensis)* extracts

Bacterial species	Type of extract aqueous (mg/mL)				Ethanolic (mg/mL)			
	C	50	100	200	C	50	100	200
Salmonella spp.	0	24	28	36	0	25	33	40
Escherichia coli	0	22	25	30	0	22	27	30
Streptococcus pyogenes	0	12	16	18	0	15	20	25
Streptococcus pneumoniae	0	15	20	27	0	14	17	21
Staphylococcus aureus	0	20	24	28	0	12	16	20

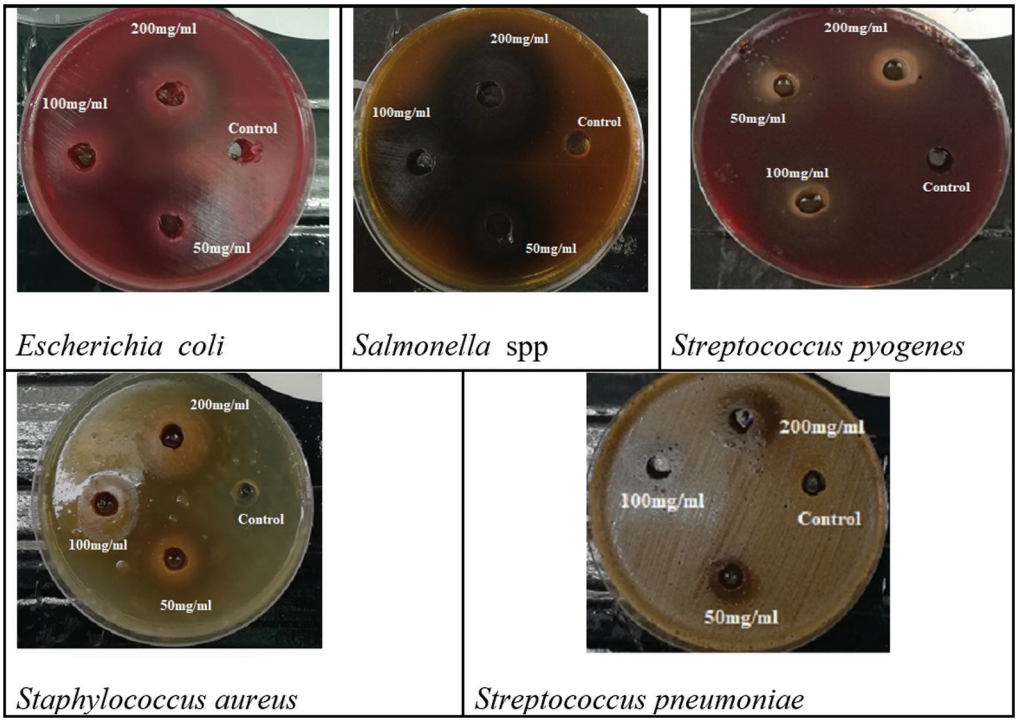

Figure 3: Antibacterial activity of green tea ethanolic extract against five bacterial species tested in the present study

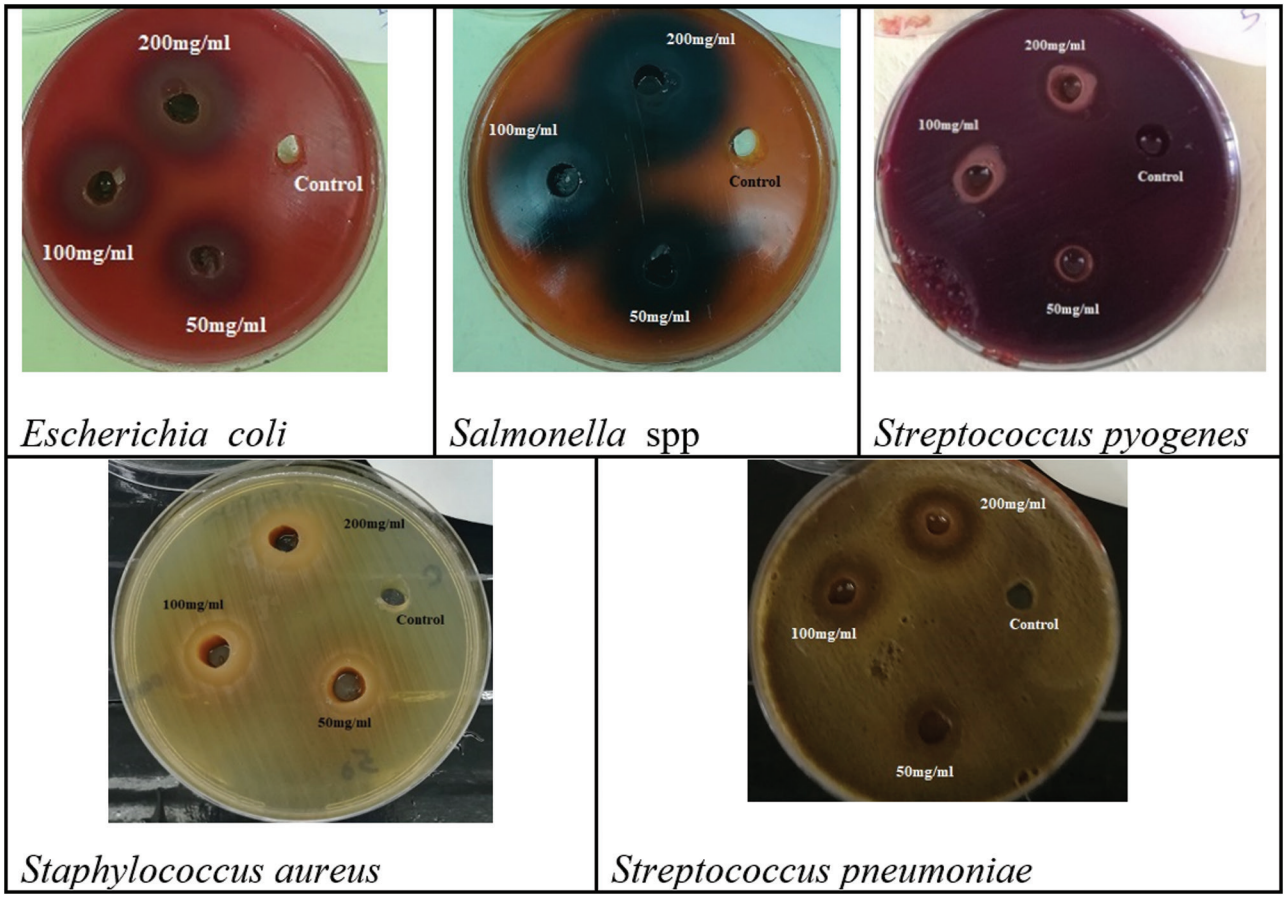

Figure 4: Antibacterial activity of green tea aqueous extract against five bacterial species tested in the present study

cancer, and malaria.[27] Plant flavonoids and phenols show antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties.[28] Tannins are utilized in antihemorrhoidal, antidiarrheal, and hemostatic formulate, while steroids have been said to have analgesic characteristics.[29]

FTIR analysis appears the presence of nine functional parts found in *C. scienensis*; these compounds have many bioactive properties, and alkanes showed cytotoxic and antibacterial properties. Functional groups like alkanes, aliphatic fluoro compounds, and metal carbonyl compounds have the responsibility for potential medicinal efficacy.[30]

A large number of constituents have been identified from the GC–MS analysis, and about 10 constituents have been reported from the analysis of *C. scienensis;* these compounds have medicinal use; propionic acid is an organic acid that acts as anti-inflammatory, antiasthmatic drugs, preservative and as an anti-microbial agent in foods produced for human and livestock consumption.[31] Flavoring agent, antifungal agent.[32] Cyclohexasiloxane and dodecamethyl serve as an emollient, de-foaming, and lubricant agents in individual care items.[33] *N*-hexadecanoic acid is utilized as a hypocholesterolemic, antioxidant, pesticidal, antiandrogenic, hemolytic, and 5-alpha reductase inhibitor.[34]

The ethanolic and aqueous green tea extracts showed clear biological activity against the studied bacterial species, whether Gram-positive or Gram-negative. These findings are in agreement with numerous other research studies, which confirmed that green tea possesses clear biological efficacy against both kinds of bacteria, Gram-positive and Gram-negative, and also against several types of viruses, in addition to being anti-inflammatory.[35-37] The antimicrobial properties of green tea could be attributed to an active compound called catechin, EGCG, which is known for its activity against dental caries and infections by multidrug-resistant *S. aureus* and human immunodeficiency virus. Numerous studies have shown that green tea's active ingredients can damage bacterial cytoplasmic membranes and also disrupt the processes of bacterial DNA replication, thus disrupting the reproduction of bacteria and inhibiting the growth of their colonies.[38,39] However, green tea has an antibacterial impact by preventing the development of biofilms.[40]

Conclusion

Various therapeutically active compounds are present in the aqueous and ethanolic extract of *C. sinensis,* which encourages its use in the treatment of microbial infections. Further investigations are needed for the chemical composition of green tea, and other techniques

like HPLC may be used. Further tests are necessary for its antimicrobial activity, either in vitro or in vivo.

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Conflicts of interest

All the authors declare no conflicts of interest otherwise disclosed in the manuscript.

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