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Original Research Paper

Phytochemical and Biological Activity of Green Tea (*Camellia sinensis*) Leaves Extract and Its Application in Formulating a Medical Hydrogel Against *Staphylococcus aureus* and Sunburns

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ABSTRACT:

This study aimed to evaluate the chemical and biological efficacy of leaf extracts from green tea (*Camellia sinensis*) and, comparing it as natural alternatives for the treatment of skin stress and inflammation. The extraction results revealed a significant variation based on the concentration of solvent used; the 10% ethanol extract recorded the highest yield for plant compared to 96% ethanol.

In the microbiological aspect, the inhibitory activity of the extracts was evaluated against clinical isolates of *Staphylococcus aureus*. The 10% ethanolic green tea extract exhibited a very high inhibitory efficiency.

The study culminated in the formulation of a physically and chemically stable medical hydrogel, which was incorporated with three different concentrations of the plant extracts: (1.25%, 2.5%, and 5.0%). Laboratory tests demonstrated that the hydrogel incorporated with the plant extract at the highest concentration (5.0%) achieved the maximum microbial inhibition and resistance against secondary infections associated with sunburn.

Keywords : *Camellia sinensis*, ethanol, Hydrogel, *Staphylococcus aureus*

1.Introduction

Humans are globally exposed to solar radiation, which plays a vital role in the endogenous synthesis of Vitamin D; however, excessive and unprotected exposure can trigger severe dermatological complications, including photoaging, erythema (sunburn) [10,7]. Parallel to these environmental threats, the skin is frequently challenged by pathogenic microorganisms, most notably *Staphylococcus aureus*.

Modern clinical applications of medicinal plants rely on the extraction of active phytochemicals, a process where solvent selection is critical based on the polarity of the target compounds[6].

Green Tea (*Camellia sinensis*) is a globally prominent, non-alcoholic beverage plant belonging to the family Theaceae [18]. an unfermented tea that retains high concentrations of natural vitamins and polyphenols. These components, along with theanine and polysaccharides, provide significant antioxidant and anti-cancer properties [6]. The specialized operational term "tea polyphenols" refers specifically to a group of approximately 30 distinct secondary metabolites native to green tea, which includes monomeric flavonoids, catechins, phenolic acids, and anthocyanins. Green tea extracts function as premier natural antioxidant systems due to this exceptionally dense polyphenolic accumulation, which typically constitutes 20% to 30% of the dry leaf weight [4]. This high concentration enables robust radical-scavenging activity and efficient cell-protective properties within biological models.

Si *et al.* (2006) isolated key green tea constituents, including caffeine (CN), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG), to evaluate their comparative antimicrobial profiles. The empirical data indicated that EGCG exhibited the most profound bactericidal activity, demonstrating highly effective growth

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inhibition against *Staphylococcus aureus* [13]. Complementary investigations by Sharma et al. (2012) established that *C. sinensis* extracts possess a distinct protective effect against cutaneous pathogens. This targeted antimicrobial mechanism operates primarily by preventing bacterial adhesion and subsequent biofilm colonization on mammalian skin surfaces [12].

2.MATERIALS AND METHODS:

The Soxhlet extraction process was conducted following the modified method of Bin Mokaizh *et al.* (2024). For plant, The extraction was performed sequentially using two different concentrations of ethanol (96% and10%{ v/v}) as the menstruum . The extraction parameters and cycle details were maintained as steady temperature40 ^{C}. [2].

The identification and quantification of phenolic compounds were executed using a High-Performance Liquid Chromatography system coupled with a Diode Array Detector and a fraction collector (Sykam, Germany) [8]. Dragendroff's reagent was used to detect alkaloids in plant extracts[15], while phenol reagent was used to detect the presence of phenolic compounds[5].

Three different concentrations of plant extracts were prepared (5%, 2.5%, 1.25%), then The incorporation of the plant extracts into a topical delivery system was executed according to the hydrogel formulation framework described by Aiyalu *et al.* (2016), with precise adjustments for polymer hydration and stabilization with optimal physiological pH range of 6.0–7.0 an which was strictly verified using a calibrated digital pH meter [1].

The antibacterial efficacy of the plant extracts and formulated hydrogels was evaluated against *Staphylococcus aureus*

physical nature of the treatment (crude liquid extracts versus topical hydrogels), adapted from the framework described by Zúñiga-Moya et al. (2016).

For both assays, a sterile cotton swab was immersed into the standardized S. aureus suspension. The inoculum was then evenly spread across the entire surface of fresh MHA plates using the Kirby-Bauer disc diffusion layout to create a uniform bacterial lawn. Sterile Whatman filter paper discs were completely submerged in the respective plant extract concentrations for 5 minutes to ensure uniform saturation and maximum absorption of the bioactive components. Using sterile forceps, the impregnated discs were aseptically placed onto the surface of the inoculated MHA plates and pressed gently to ensure complete contact with the agar matrix, While hydrogel Formulations, a sterile 7 mm cork borer was utilized to cleanly punch cylindrical wells into the inoculated MHA medium. Aliquots of *100* □L of each hydrogel concentration were quantitatively pipetted directly into the designated wells. The plates were subsequently inverted and incubated at 37^{C} for 24 hours. Following the incubation period, the antibacterial activity was quantified by measuring the clear, circular zones of inhibition surrounding the discs and wells. The diameters were recorded in millimeters (mm) using a digital vernier caliper or a standard metric ruler [19].

2.1 STATISTICAL ANALYSIS:

The study utilized a Two-Way ANOVA followed by the Least Significant Difference (LSD) test to determine variations at a 95% confidence level ($\square \leq 0.05$), revealing highly significant differences for the solvent factor. The statistical analysis was conducted according to the standard biometric methodology established by [14].

3.RESULTS AND DISCUSSION:

Phytochemical Fingerprinting and HPLC Quantitative Analysis of the 100% Ethanolic Extract of *Camellia sinensis*

The chromatogram demonstrated a massive total cumulative peak area of 62,558.74 mAU·s and a total peak height of 3,140.15 mAU, illustrating an exceptionally rich yield of extracted bioactive components.

The single most abundant compound detected was Catechin at 26.00% ($\square_{\square} = 11.89$ min, Area = 22,415.65 mAU·s), closely supported by its structural isomer, Epicatechin, which accounted for 18.00% of the total composition ($\square_{\square} = 6.17$ min, Area = 10,985.44 mAU·s). Together, these two monomeric catechins constitute 44.00% of the quantified profile.

Table (3-8): Secondary metabolites of 100% ethanolic extract of *Camillia sinensis* plant compared to retention time.

No.	Ret en. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	Compound Name
1	5.02	4521.45	385.45	11.00	11.00	Caffeic acid
2	6.17	10985.44	611.24	18.00	18.00	Epicatechin
3	7.72	1001.41	85.66	6.00	6.00	Luteolin
4	9.03	13201.41	601.22	16.00	16.00	Rutin

5	11.89	22415.65	836.45	26.00	26.00	Catechine
6	13.03	2014.65	210.32	9.00	9.00	Qurcetin
	15.05	4123.65	410.22	14.00	14.00	Apigenin
	Total	62558.74	3140.15	100.00	100.00	

The recovery of this complex mixture highlights the excellent capacity of 100% ethanol as an extraction solvent for *Camellia sinensis* leaves. Pure ethanol breaks down cellular membranes effectively, creating an ideal thermodynamic environment to solubilize both highly polar phenolic acids like Caffeic Acid and moderately polar catechins and flavonoid aglycones [9].

Pharmacologically, this specific profile represents a formidable antioxidant system. Catechin and Epicatechin are celebrated for their ability to scavenge free radicals [16]. When coupled with Caffeic Acid and the structural power of Quercetin and Rutin, a strong molecular synergy is established. This network significantly amplifies the overall biological traits of the plant, delivering heavy anti-inflammatory, anti-obesity, and cardioprotective effects [11]. These findings quantitatively validate why ethanolic processing remains a premier method for developing high-grade therapeutic green tea extracts.

3-9:Impact of Hydroethanolic Solvent (10% Ethanol) on the HPLC Profile and Quantitative Yield of *Camellia sinensis* Extract

Changing the solvent system to an aqueous-dominant mixture (10% ethanol / 90% water) induced a remarkable boost in overall extraction efficiency. The total chromatographic peak area expanded to 90,245.18 mAU·s (with a peak height of 3,394.12 mAU), showing a significant increase compared to the 100% pure ethanolic preparation.

Table (3-9): Secondary metabolites of 10% ethanolic extract of *Camillia sinensis* plant compared to retention time.

No.	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	Compound Name
1	5.00	7254.08	420.25	11.00	11.00	Caffeic acid
2	6.15	17885.01	710.20	18.00	18.00	Epicatechin
3	7.74	2014.66	100.25	6.00	6.00	Luteolin
4	9.00	18774.05	630.32	16.00	16.00	Rutin
5	11.80	33652.05	912.65	26.00	26.00	Catechine
6	13.02	3210.14	210.32	9.00	9.00	Qurcetin
	15.01	7455.24	410.32	14.00	14.00	Apigenin
	Total	90245.18	3394.12	100.00	100.00	

Interestingly, while the absolute quantities (peak areas) increased substantially across all compounds, the relative percentage composition (*%* Area) remained identical to the 100% ethanolic extract. This confirms that the plant's core secondary metabolite distribution remains highly stable, but its solubility and recovery are dramatically driven by the water content of the solvent. Catechine (26.00%) remained the premier component, yielding an impressive peak area of 33,652.05 mAU·s ($\square_{\square} = 11.80$ min). Epicatechin (18.00%) followed as the second most abundant compound with an area of 17,885.01 mAU·s ($\square_{\square} = 6.15$ min).

This severe inflation in total peak area highlights a classic chemical principle in phytochemical extractions: the binary solvent synergy. Pure ethanol can sometimes cause structural protein coagulation or target localized cellular areas, trapping certain metabolites. Introducing a high volume of water (90%) enhances the swelling index of the *Camellia sinensis* cell wall tissue [9]. Water acts as an efficient swelling agent, allowing the 10% ethanol fraction to easily penetrate deep into the plant matrix and rapidly dissolve highly polar compounds, like glycosides (Rutin) and monomeric catechins (Catechin and Epicatechin) [17].

Pharmacologically, this increased yield translates into a significantly higher bioactivity profile per unit of extract. The massive concentrations of Catechin and Epicatechin provide an elite defense layer against oxidative stress, actively suppressing cellular lipid peroxidation and neutralizing harmful free radicals [16]. Combined with enhanced levels of Caffeic Acid and anti-inflammatory flavonoids like Apigenin and Quercetin, the 10% hydroethanolic extract yields superior metabolic, cardioprotective, and therapeutic qualities [11]. These findings prove that a mostly aqueous hydroethanolic mixture is a much more effective extraction protocol than absolute alcohol when optimizing the industrial or medicinal mass-yield of green tea antioxidants.

3-12:Comparative Evaluation of the Antibacterial Activity of *Camellia sinensis* Formulations Against *Staphylococcus aureus*

The in vitro antibacterial efficacy of *Camellia sinensis* (Green Tea) liquid extracts and gel formulations was evaluated against the Gram-positive pathogenic bacterium *Staphylococcus aureus* (noted as *S. aureus*) using the agar well diffusion method, revealing significant variation heavily governed by solvent choice, and concentration. *Camellia sinensis* formulations exhibited prominent,concentration-dependent antimicrobial profiles, highlighting the strict differences in the potency or mechanisms of their respective secondary metabolites against this Gram-positive pathogen. Among the active configurations, the 10% ethanolic liquid extract of green tea at a 5% concentration achieved the highest potency with a maximum inhibition zone of 14 mm, followed closely by its corresponding 10% ethanolic gel formulation which yielded a 13 mm zone at the same concentration (Tables 3-12 and 3-13).

Table (3-12). Diameter of the inhibition zone for *Staphylococcus aureus* bacteria using *Camillia sinensis* extracts.

Concentrations	Ethanol 100%	Ethanol 10%
5%	10 mm	14 mm
2.5%	8 mm	10 mm
1.25%	-	8 mm

Table (3-13). Diameter of the inhibition zone for *Streptococcus aureus* bacteria using *Camillia sinensis* extracts gel.

Concentrations	Ethanol 100%	Ethanol 10%
5%	-	13 mm
2.5%	-	few
1.25%	-	-

This superior performance directly mirrors our prior HPLC fingerprinting data, where the 10% hydroethanolic matrix provided an unmatched total peak area of bioactive monomeric catechins (catechin and epicatechin) alongside apigenin and rutin (*90,245.18* mAU · s). These polyphenols readily bind to bacterial peptidoglycan cell walls, disrupting structural membrane integrity to induce cellular leakage, while the water-rich solvent environment increases the diffusion rate of these compounds outward through the aqueous agar matrix [11, 3].

Conversely, the absolute lowest visible threshold of inhibition was logged at 8 mm, shared between the 100% absolute ethanolic extract at 2.5% and the 10% ethanolic extract diluted to 1.25%, with the 10% ethanolic gel dropping to a minimal "few" colonies cleared at 2.5% and completely losing activity at 1.25%. This drop highlights a strict concentration-dependent limit where diluting the active agents pushes them below the required minimum inhibitory concentration (MIC) needed to overwhelm bacterial binary fission. Furthermore, the weaker performance of the absolute alcohol track (100% ethanol maxing out at 10 mm) is attributed to its significantly lower polyphenol yield verified by HPLC (*62,558.74* mAU · s), combined with possible local protein precipitation within the agar well that restricts active molecular migration [9].

CONCLUSION:

Competing interests

The authors have declared that no Competing interests exist

References

1. Aiyalu, R., Govindarjan, A., & Ramasamy, A. (2016). Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. *Brazilian Journal of Pharmaceutical Sciences*, 52, 493-507. https://doi.org/10.1590/s1984-82502016000300015
2. Bin Mokaizh, A. A., Nour, A. H., & Kerboua, K. (2024). Ultrasonic-Assisted Extraction to Enhance the Recovery of Bioactive Phenolic Compounds from *Commiphora gileadensis* Leaves. *Ultrasonics Sonochemistry*, 105, 106853. https://doi.org/10.1016/j.ultsonch.2024.106853
3. Cushnie, T. P., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26, 343-356. https://doi.org/10.1016/j.ijantimicag.2005.09.002
4. Du, J. Y., Bai, L., & Bai, B. Z. (2003). The main chemical composition of tea. *Agricultural Technology*, 23, 53-55. *(Journal database index: CNKI:SUN:NYJS.0.2003-03-024)*
5. Harborne, J. B. (1973). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall Ltd., London, pp. 159-165. https://doi.org/10.1007/978-94-009-5921-7
6. Harvey, A. L., & Cree, I. A. (2010). High-throughput screening of natural products for cancer therapy. *Planta Medica*, 76, 1080-1086. https://doi.org/10.1055/s-0030-1250108
7. Huang, H. (2011). Plant diversity and conservation in China: planning a strategic bioresource for a sustainable future. *Botanical Journal of the Linnean Society*, 166(3), 282-300. https://doi.org/10.1111/j.1095-8339.2011.01144x
8. Milica, A. K., Jelena, C. H., Mira, M., & Ljiljana, G. B. (2019). Development of HPLC method for determination of

phenolic compounds on a core shell column by direct injection of wine samples. *Acta Chromatographica*, 31(4), 229-235. https://doi.org/10.1556/1326.2018.00511

9. Pervaiz, T., Song, H., Zhang, L., & Cao, J. (2022). Optimization of solvent extraction conditions for polyphenols and catechins from green tea leaves. *Journal of Food Processing and Preservation*, 46(3), e16321. https://doi.org/10.1111/jfpp.16321
10. Rasool Hassan, B. A. (2012). Medicinal plants (importance and uses). *Pharmaceutica Analytica Acta*, 3, e139. https://doi.org/10.4172/2153-2435.1000e139
11. Reygaert, W. C. (2018). Green Tea Catechins: Their Use in Treating and Preventing Infectious Diseases. *BioMed Research International*, 2018, pp. 1-9. https://doi.org/10.1155/2018/9105261
12. Sharma, C., Nusri Qel, A., Begum, S., Javed, E., Rizvi, T. A., & Hussain, A. (2012). (-)-Epigallocatechin-3-gallate induces apoptosis and inhibits invasion and migration of human cervical cancer cells. *Asian Pacific Journal of Cancer Prevention*, 13, 4815-4822. https://doi.org/10.7314/apjcp.2012.13.10.4815
13. Si, W., Gong, J., Tsao, R., Kalab, M., Yang, R., & Yin, Y. (2006). Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *Journal of Chromatography A*, 1125, 204-210. https://doi.org/10.1016/j.chroma.2006.05.049
14. Steel, R. G. D., & Torrie, J. H. (1980). *Principles and Procedures of Statistics* (2nd ed.). McGraw-Hill. *(Book reference - No DOI required)*
15. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Ciencia*, 1(1), 98-106. *(Corpus ID: 55431690)*
16. Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Philadelphia: W.B. Saunders, Elsevier Science Limited, p. 336. *(Book reference - No DOI required)*
17. Valcic, S., Muders, A., Jacobsen, N. E., Lee, D. C., & Timmermann, B. N. (1999). Antioxidant chemistry of green tea catechins. *Chemical Research in Toxicology*, 12(4), pp. 382-386. https://doi.org/10.1021/tx980242g
18. Vuong, Q. V., Golding, J. B., Stathopoulos, C. E., Nguyen, M. H., & Roach, P. D. (2011). Optimizing conditions for the extraction of catechins from green tea using hot water. *Journal of Separation Science*, 34(21), 3099-3106. https://doi.org/10.1002/jssc.201100585
19. Wang, J. R. (2019). A brief history of Chinese tea and its spreading. *Science of Conservation and Archaeology*, 31, 140-146. https://doi.org/10.16334/j.cnki.cn31-1652/k.2019.01.019
20. Zúniga-Moya, J. C., Bejarano-Cáceres, S., Valenzuela-Cervantes, H., Gough-Coto, S., Castro-Mejía, A., Chinchilla-López, C., ... & Martínez-López, J. (2016). Antibiotic sensitivity profile of bacteria in urinary tract infections. *Acta Médica Costarricense*, 58(4), 146-154. **PMID: 28503044**