Research Article

Extraction, Phytochemical Investigation and Comparative Antimicrobial Study of Lemon Grass and Holy Basil Leaves Extracts

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ABSTRACT

This study investigates the antibacterial properties of two traditional plants—Lemon Grass (*Cymbopogon citratus*) and Holy Basil (*Ocimum sanctum*) through solvent extractions to evaluate their combined synergistic effect against selected Gram-positive bacterial strains using the agar well diffusion method. Ethanol, hot water, and cold-water extracts of both plants were prepared and subjected to phytochemical screening. Active constituents such as flavonoids, tannins, phenols, saponins, glycosides, and terpenoids were identified in varying concentrations across the extracts. Antibacterial activity was measured by comparing the zone of inhibition of each extract and its combinations against *Staphylococcus aureus*, using Penicillin G as the reference standard. The study's findings revealed that the hot water extract of Holy Basil exhibited the highest antibacterial activity, as indicated by the zone of inhibition (ZOI: 0.8 cm), followed by the cold water and ethanol extracts. Both the extracts (ethanol and cold water) of Lemon Grass showed moderate activity (ZOI: 0.5 cm). Synergistic testing of extract combinations demonstrated that Lemon Grass: Holy Basil (1:2) ethanol extract produced a higher inhibition zone (0.6 cm) than either of the extracts alone, suggesting a synergistic interaction between possible citral and eugenol-rich phytochemicals. This study presents an insight into the combination of phytomedicines and supports the future integration of the *C. citratus* and *O. sanctum* plants or their parts or various extracts in different combinations of phytopharmaceutical antibacterial formulations to test their efficacy through *in vivo* validation and mechanistic exploration.

Keywords: Lemon Grass, Holy Basil, Antibacterial activity, Zone of Inhibition, Herbal extracts.

INTRODUCTION

The emergence of antibiotic-resistant microorganisms is a global health crisis with serious implications for clinical medicine, food security, and public health [1]. Bacteria, although vital to ecosystems and human health, are responsible for a wide range of infections. The World Health Organization (WHO) has listed antimicrobial resistance (AMR) as one of the top 10 global public health threats facing humanity [2]. An alarming rise in multidrug-resistant (MDR) strains of pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* has rendered many first-line antibiotics ineffective [3]. Antibiotics were once considered miracle drugs, but their overuse in both healthcare and agriculture has contributed to the accelerated evolution of resistance mechanisms in bacteria [4]. The lack of new antibiotic classes further exacerbates the situation [5].

As conventional antibiotics fail, there is a growing interest in plant-based antimicrobials, which offer multi-target modes of action, minimal side effects, and low potential for resistance development [6, 7]. Plants have long been used in traditional medicine to treat microbial infections. Phytochemicals like flavonoids, alkaloids, terpenoids, phenolic compounds, and essential oils play a significant role in plant defence and have demonstrated antimicrobial activity against a wide spectrum of bacteria [8, 9]. These natural compounds act through multiple mechanisms, including disruption of bacterial membranes, enzyme inhibition, and interference with DNA replication [10].

Cymbopogon citratus (Lemon Grass) is a tropical aromatic grass that belongs to the family Poaceae. It is traditionally used in teas, soups, and medicinal preparations across Asia and Africa [11]. Its essential oil contains citral—a mix of the isomers neral and geranial—which exhibits strong antibacterial, antifungal, and anti-inflammatory effects [12].

The mechanism involves membrane disruption and interference with bacterial quorum-sensing pathways [13]. Studies have shown its effectiveness against *S. aureus*, *E. coli*, and other Gram-positive strains [14, 15].

Ocimum sanctum (Holy Basil), also known as Tulsi in Ayurveda, is a revered plant in India with both spiritual and medicinal significance. Belonging to the family Lamiaceae, it contains key bioactive compounds such as eugenol, ursolic acid, and rosmarinic acid [16]. Eugenol has been shown to disrupt bacterial membranes, inhibit enzymes, and induce oxidative stress in pathogens [17].

O. sanctum extracts exhibit antibacterial, antiviral, antioxidant, and anti-inflammatory properties, making it suitable for treating respiratory and gastrointestinal infections [18, 19]. This study aims to evaluate the antibacterial efficacy of individual and combined extracts of *C. citratus* and *O. sanctum* prepared using ethanol, cold water, and hot water. The results will contribute to the growing field of phytomedicine by identifying potential plant-based alternatives to synthetic antibiotics.

MATERIALS AND METHODS

This study employed dried leaf samples of *Ocimum sanctum* (Holy Basil) and *Cymbopogon citratus* (Lemon Grass), which were collected from local markets in Lucknow, India. Analytical-grade solvents and reagents were used for extraction and phytochemical screening. All glassware and equipment were sterilised before use.

Reagents and Chemicals: Ethanol (95%), Distilled water, Dragendorff's reagent, Dilute NaOH and HCl, Ferric chloride (1% and 5%), Chloroform, Concentrated sulfuric acid, Glacial acetic acid, Iodine solution, Nutrient agar, etc. *Staphylococcus aureus* strains (MTCC No 3160) were procured from Microbial Type Culture Collection (MTCC), at Institute of Microbial Technology (IMTECH), Chandigarh, India, in freeze-dried form, and were cultured as per standard protocol.

Equipment: Mixer Grinder, Reflux setup and steam distillation apparatus, Weighing Balance, B.O.D. Incubator, Laminar Air Flow Chamber, Water Bath, Petri Dishes, Inoculation Loop, Autoclave and Sterile Pipettes

Sample Preparation: Leaves of both plants were washed thoroughly and dried at room temperature, then oven-dried at 45°C. The dried material was ground into a coarse powder using a sterile grinder and stored in airtight containers until extraction.

Preparation of Plant Extracts: Three types of extracts, *viz.,* **ethanol, cold water,** and **hot water**, were prepared for both *O. sanctum* and *C. citratus*.

- ***Ethanol Extract****:* 25 g of powdered sample was soaked in 100 mL of ethanol for 24 hours, followed by reflux and steam distillation.
- ***Cold Water Extract****:* 25 g of powder soaked in 100 ml of distilled water at room temperature for 24 hours.
- ***Hot Water Extract****:* Same as above, but water heated to 80°C during soaking to enhance extraction efficiency[20].

Preliminary Phytochemical Screening: Standard phytochemical tests were carried out to detect the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides, and essential oils using protocols from Shihata (1951) and later standardised procedures [21, 22].

Preparation of Agar Media: Nutrient agar media was prepared using peptone, beef extract, sodium chloride, agar, and distilled water. The medium was autoclaved at 121°C for 15 minutes, poured into sterile Petri dishes, and allowed to solidify.

Antibacterial Testing - Agar Well Diffusion Method*:* The Agar Well Diffusion Method was used to evaluate antibacterial activity against *Staphylococcus aureus* [23].

- **Inoculum Preparation**: Overnight broth cultures were spread evenly over the agar surface.
- **Well Creation**: Wells of 6 mm diameter were punched using a sterile cork borer.
- **Application**: 100 µL of each plant extract was introduced into the designated wells. Control wells contained solvents (ethanol or water), and a standard well with Penicillin G (10 µg/mL) as a positive control.
- **Incubation**: Plates were incubated at 37°C for 24 hours.
- **Measurement**: Zones of inhibition (ZOI) were measured in centimetres using a Vernier calliper.

Synergistic Testing: Plant extracts were combined in a 1:2 ratio (Lemon Grass: Holy Basil and vice versa) and applied using the same agar well method to assess synergy. Zones of inhibition were compared with those of individual extracts as well as with the standard control [24].

Data Analysis: Each plant extract's mean zone of inhibition diameter was determined and compared. The statistical analysis was done by calculation of the mean ± SD among the results using two-way ANOVA followed by Tukey's test. All the Statistical data were calculated using the software GraphPad Prism Ver. 8.02.

RESULTS

In the present study, antibacterial efficacy of ethanol, hot water, and cold-water extracts of *Ocimum sanctum* (Holy Basil) and *Cymbopogon citratus* (Lemon Grass), both individually and in combination, against *Staphylococcus aureus was evaluated* using the agar well diffusion method. The zone of inhibition (ZOI) was measured in centimetres to determine antimicrobial activity.

Table 1: Phytochemical Composition of Extracts

Phytoconstituents	*O. sanctum*			*C. citratus*		
	Ethanol	Hot Water	Cold Water	Ethanol	Hot Water	Cold Water
Alkaloids	Present	Present	Present	Present	Present	Present
Flavonoids	Present	Present	Present	Present	Present	Present
Saponins	Present	Present	Present	Present	Present	Absent
Tannins	Present	Present	Present	Present	Present	Absent
Phenols	Present	Present	Present	Present	Present	Present
Terpenoids	Present	Present	Absent	Present	Absent	Absent
Glycosides	Present	Present	Present	Present	Present	Present

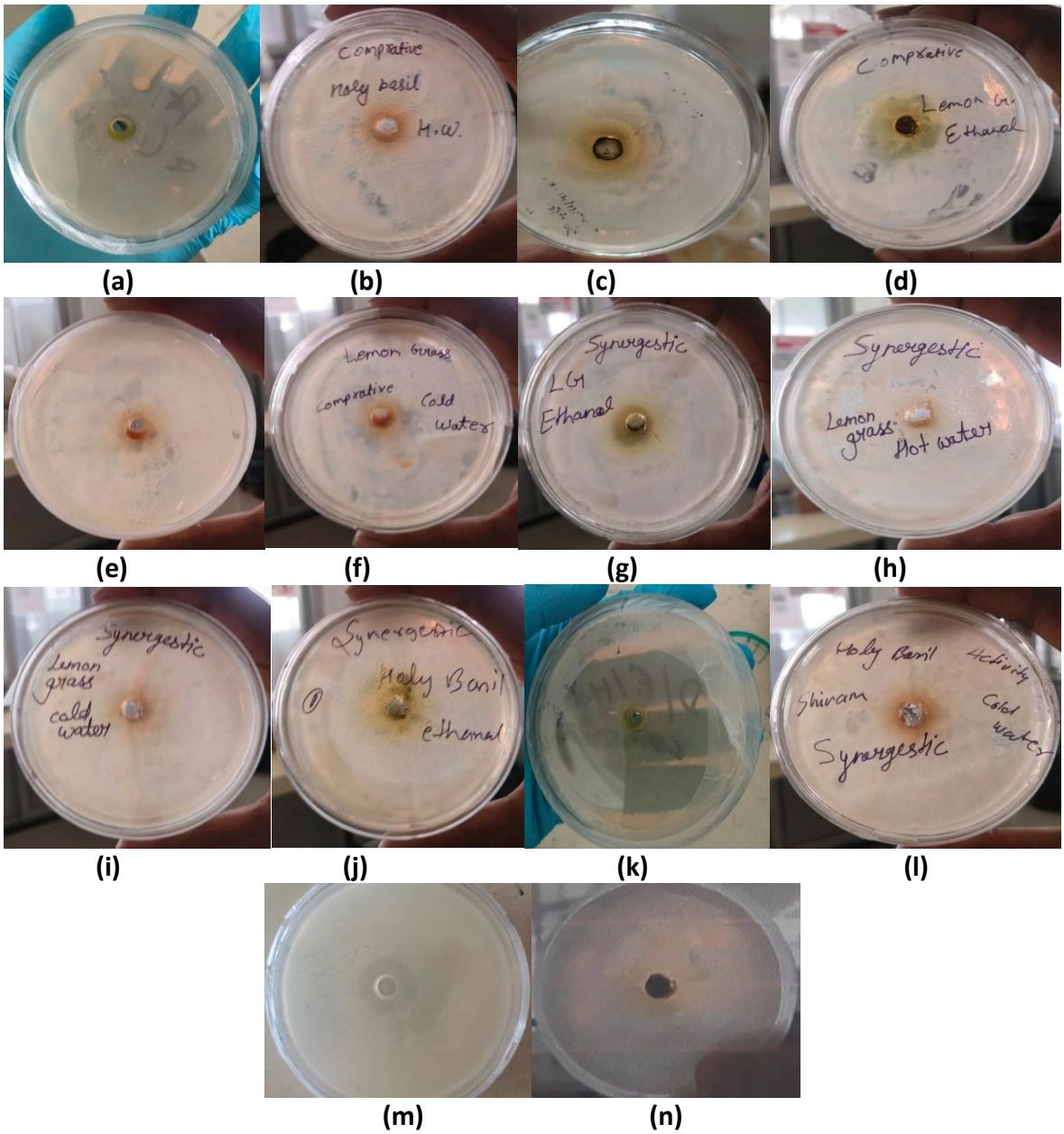

Figure 1: Different plates of the samples: a. Comparative Activity Holy Basil Ethanol Extract, b. Comparative Activity Holy Basil Hot Water Extract, c. Comparative Activity Holy Basil Cold Water Extract, d. Basil Comparative Activity Lemon Grass Ethanol Extract, e. Comparative Activity Lemon Grass Hot Water Extract, f. Comparative Activity Lemon Grass Cold Water Extract, g. Synergistic Activity Lemon Grass Ethanol Extract, h. Synergistic Activity Lemon Grass Hot Water Extract, i. Synergistic Activity Lemon Grass Cold Water Extract, j. Synergistic Activity Holy Basil Ethanol Extract, k. Synergistic Activity Holy Basil Hot Water Extract, l. Synergistic Activity Holy Basil Cold Water Extract, m. Standard drug (Penicillin G), n. Control

Preliminary Phytochemical Screening: Qualitative phytochemical tests revealed the presence of major bioactive compounds across different extracts, as shown in Table 1. Holy Basil showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, and glycosides in all extracts, while terpenoids were absent in its cold-water forms. Lemon Grass displayed a similar profile, though the presence of alkaloids, flavonoids, phenols and glycosides in all extracts, while saponins and tannins were absent in its cold-water forms, whereas terpenoids were absent in both hot-water and cold-water forms. These secondary metabolites are known for their antibacterial properties. Flavonoids and tannins disrupt bacterial cell membranes, alkaloids interfere with DNA synthesis, and phenols act as oxidising agents [25, 26]. Terpenoids and glycosides may exert synergistic antimicrobial effects when combined with other classes.[27]

Zone of Inhibition (ZOI) Results: The ZOI assay quantitatively confirmed the antibacterial activity of individual and combined plant extracts, as shown in Table 2. All measurements were conducted in triplicate, and average values were taken. Penicillin G served as a positive control and as a reference standard, as shown in Figure 1.

Individual Extracts: Among the *O. sanctum* extracts, the hot water extract exhibited the highest antibacterial activity

with a ZOI of 0.8 cm, followed by the cold-water extract (0.6 cm) and the ethanol extract (0.2 cm), as shown in Figure 2. For *C. citratus*, the ethanol and cold-water extracts showed ZOIs of 0.5 cm, while the hot water extract recorded the lowest activity (0.4 cm), as shown in Figure 3.

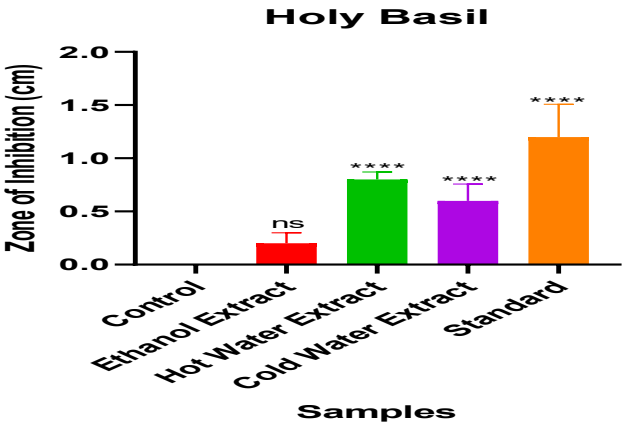

Figure 2: Different extracts of Holy Basil. All data were analysed *via* two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests using GraphPad Prism software. Data presented are Mean ± SD (n =5). ns P values are considered non-significant, ****P<0.0001 when compared to the control group.

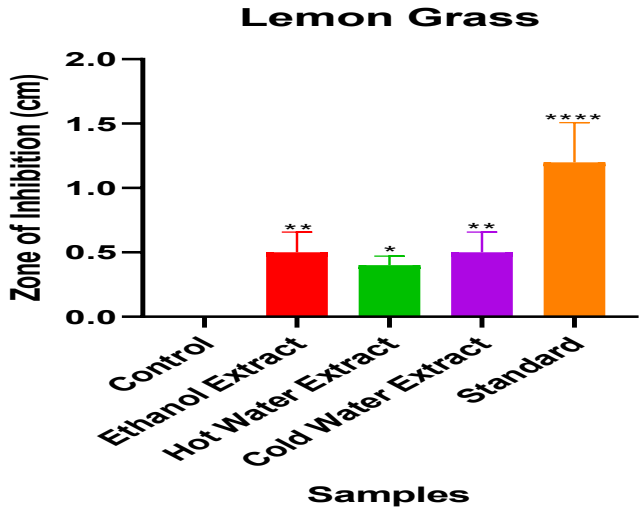

Figure 3: Different extracts of lemon grass. All data were analysed *via* two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests using GraphPad Prism software. Data presented are Mean ± SD (n =5). ****P<0.0001, **P<0.01 and *P<0.1 when compared to the control group.

Combined Extracts (Synergistic Evaluation): When extracts were combined in a 1:2 ratio (Lemon Grass: Holy Basil and vice versa), results suggested partial synergistic effects, as shown in Figure 4 and Figure 5. The ethanol-based combination (Lemon grass: Holy Basil) yielded the highest ZOI at 0.6 cm, exceeding individual ethanol extract activity. The hot and cold-water combinations showed moderate results (0.3–0.5 cm), with no extract combination surpassing the hot water extract of Holy Basil used alone.

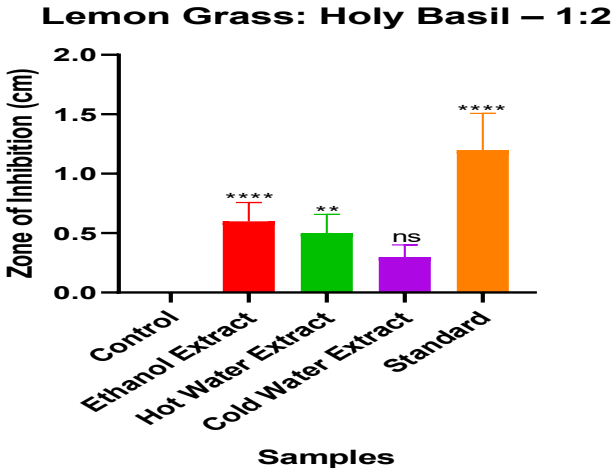

Figure 4: Different extracts of Lemon Grass: Holy Basil – 1:2. All data were analysed *via* two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests using GraphPad Prism software. Data presented are Mean ± SD (n =5). ns P values are considered non-significant, ****P<0.0001, ***P<0.001, and **P<0.01 when compared to the control group.

Table 2: Zone of Inhibition (ZOI) by Sample Type

S. No	Sample Description	ZOI (cm)
1	Control	0.0
2	Holy Basil – Ethanol Extract	0.2
3	Holy Basil – Hot Water Extract	0.8
4	Holy Basil – Cold Water Extract	0.6
5	Lemon Grass – Ethanol Extract	0.5
6	Lemon Grass – Hot Water Extract	0.4
7	Lemon Grass – Cold Water Extract	0.5
8	Lemon Grass: Holy Basil – Ethanol (1:2)	0.6
9	Lemon Grass: Holy Basil – Hot Water (1:2)	0.5
10	Lemon Grass: Holy Basil – Cold Water (1:2)	0.3
11	Holy Basil: Lemon Grass – Ethanol (1:2)	0.4
12	Holy Basil: Lemon Grass – Hot Water (1:2)	0.4
13	Holy Basil: Lemon Grass – Cold Water (1:2)	0.3
14	Penicillin G (Standard)	1.2

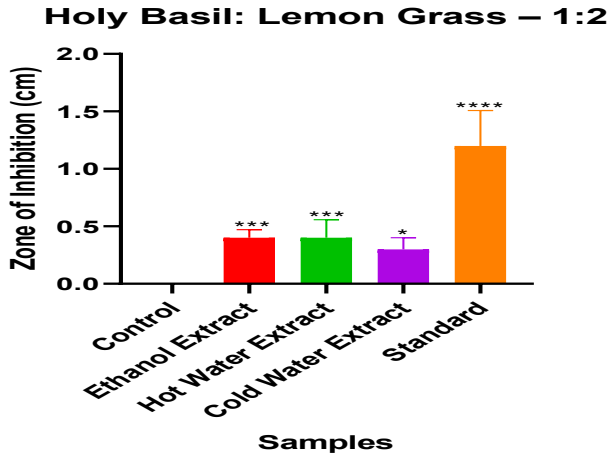

Figure 5: Different extracts of Holy Basil: Lemon Grass – 1:2. All data were analysed *via* two-way analysis of variance

(ANOVA) followed by Tukey's multiple comparisons tests using GraphPad Prism software. Data presented are Mean ± SD (n =5). ****P<0.0001, ***P<0.001 and *P<0.1 when compared to the control group.

DISCUSSION

The highest antimicrobial activity was observed in the hot water extract of *O. sanctum* (0.8 cm), suggesting that water-soluble compounds such as phenols, tannins, and alkaloids are more extractable at elevated temperatures. This is consistent with previous research showing that *O. sanctum* aqueous extracts contain phenolic acids and flavonoids with significant antimicrobial activity [28].

Conversely, *C. citratus* extracts showed moderate antibacterial effects, aligning with studies that highlight citral's potency but also its thermal instability [29]. The slightly reduced activity of hot water extracts from Lemon Grass supports this, as excessive heat may degrade citral and other thermolabile components [30]. The combination of ethanol extracts from Lemon Grass and Holy Basil showed improved activity (0.6 cm) compared to the individual extracts, indicating possible synergistic effects between citral and eugenol. Similar synergism has been documented when combining essential oils containing aldehydes and phenylpropanoids [31, 32]. However, combinations prepared in water did not exhibit enhanced activity, possibly due to dilution or compound incompatibility. Though all plant-based extracts showed lower activity than Penicillin G (1.2 cm), their multi-target action and phytochemical diversity could offer longer-term benefits in reducing bacterial resistance development [33].

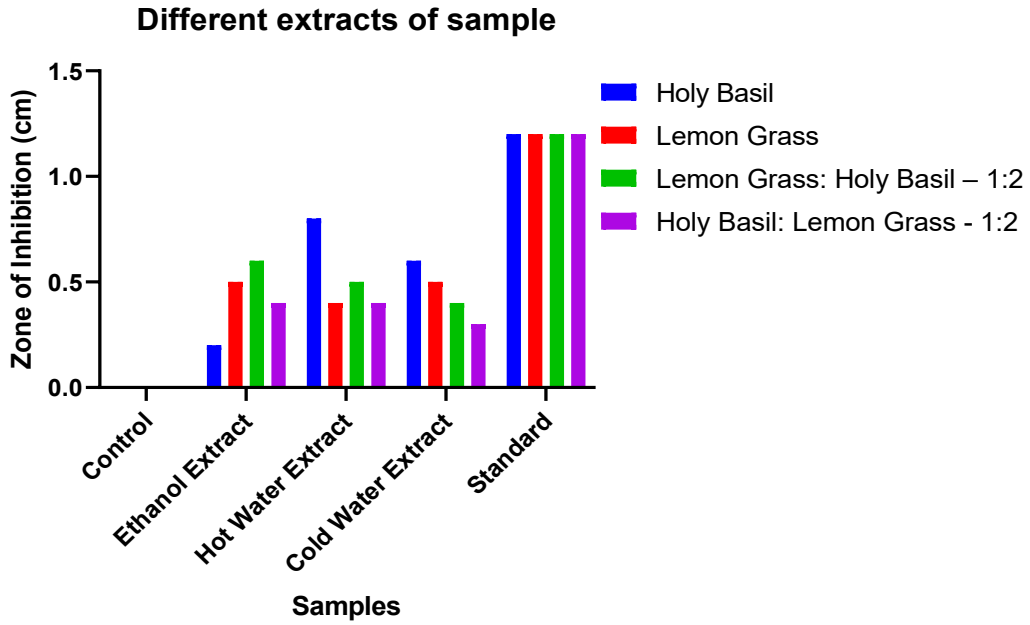

Figure 6: Different extracts of the sample

CONCLUSION

This study provides strong preliminary evidence for the antibacterial potential of *Ocimum sanctum* and *Cymbopogon citratus* extracts, especially when extracted using hot water and ethanol. The combination approach also highlights the potential for synergistic enhancement, though results varied based on the extraction method and ratio. This study supports the future integration of *C. citratus* and *O. sanctum* into phytopharmaceutical antibacterial formulations and encourages further *in vivo* validation and mechanistic exploration.

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