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# Impact of different extraction solvents and concentrations on the total phenolics content and bioactivity of the Algerian lemongrass (*Cymbopogon citratus*) extracts

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**Abstract.** Due to its widespread range and great variety of applications, *Cymbopogon citratus* (DC. ex Nees) Stapf. (Poaceae) is one of the most commercially important plants in the world. In this investigation, for the proper analysis of the phenolic compounds present in plant tissues, it is essential to understand how solvent concentration and type affect the extraction process. Three different extraction solvents such as acetone, ethanol, and methanol were used at different concentrations ranging from 20% to 100% each to assess both the total phenolic (TPC) and total flavonoid content (TFC) present in each extract of *C. citratus*. Antibacterial activity against Gram-positive and Gram-negative bacteria, as well as two different types of fungus, was evaluated using the disc diffusion method. The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was used as a proxy for antioxidant capacity. The antimicrobial activity results showed that the acetone 60% extract was most effective against *Pseudomonas aeruginosa* (IZ = 17 mm), while the acetone 80% extract was more effective against *S. cerevisiae* (IZ = 18 mm). When tested against *Candida albicans* and *Saccharomyces cerevisiae*, the highest effective antifungal activity was found in the 80% acetone and 80% methanol extracts, respectively. The highest DPPH-RSA $IC_{50}$ value reported was 19.22 for ethanol at 60% concentration which is correlated mainly to its greatest total flavonoid content (58.7 mg QE/100 g) in addition to (300.1 mg GAE/100 g) as a TPC followed by acetone (80%) with $IC_{50}$ value 21.16. A quantitative analysis revealed that the greatest concentrations of polyphenolic compounds were found in 80% acetone (370.2 mg GAE/100 g) and 60% acetone (353.9 mg GAE/100 g), while the greatest values for total flavonoid concentration were found in 60% ethanol (58.7 mg QE/100 g) followed by 80% and 60% methanol, with 57.1 and 55.2 mg QE/100 g, respectively. In conclusion, the plant under study included a number of bioactive compounds that may be put to use in a range of unique medical and aesthetic preparations.

***Keywords*****:** *Cymbopogon citratus;* Poaceae; antioxidants; antimicrobial activity; antifungal agents; flavonoids.

## 1. Introduction

The use of medicinal plants in traditional medical systems has garnered considerable attention owing to the diverse array of chemical constituents found in their purified extracts, which possess notable pharmacological and clinical attributes [1]. Notably, medicinal plants contain chemical constituents with potent antioxidant activity, which are linked to their ability to prevent different degenerative illnesses such as cancer, neurological problems, and heart disease [2]. The use of medicinal plants as the foundation of healthcare systems in many societies has gained significant recognition. The recovery and preservation

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of traditional knowledge associated with plant resources are integral to biodiversity conservation, the growth of novel drugs, and the enhancement of individual quality of life, especially in neglected regions. Furthermore, the growth of multidrug-resistant bacteria and the negative side effects associated with existing antibiotics have driven the hunt for innovative plant-derived antimicrobial medicines [3]. Many kinds of plants have been found for their therapeutic characteristics, which include antioxidant, anti-inflammatory, antibacterial, hypolipidemic, anti-mutagenic, and anti-carcinogenic capabilities [4]. Antimicrobial activity has been demonstrated in extracts taken from many plant components, including leaves, flowers, fruits, and herbs. However, many plant extracts' particular methods of action are still unknown [5]. Lemongrass (*Cymbopogon citratus*) is a popular medicinal herb with several uses. Traditionally, lemongrass has been used as a sedative, fever treatment, and indigenous remedy for infectious diseases. Additionally, it has been employed externally to address skin conditions such as ringworms, athlete's foot, and scabies. Because of its capacity to control excessive oil glands, it is a potential toning astringent for oily skin, and it has even been used to treat pimples, acne, and blackheads. Furthermore, lemongrass is thought to be useful for the treatment of bugs and dandruff [6].

Of particular interest is lemongrass's potential in cancer research. Animal studies have demonstrated that lemongrass oil may possess preventive properties against colon cancer and other types of cancer. When reactive oxygen species (ROS) exist in significant amounts and for an extended period of time, the body enters an oxidative stress state, producing damage to cells that manifests in a variety of conditions such as cardiac and neurological conditions, cancer, and overall inflammation. The use of external antioxidants aids in the reduction of such effects. Many antioxidant activity experiments have revealed that methanol and methanol/water extracts, injections, and decoctions of *Cymbopogon citratus*, notably the phenolic division, have the potential to remove ROS [7, 8].

It has been suggested that the antioxidant activity that exists in plant polyphenol percentages may be a key factor in vascular dysfunctions caused by oxidative stress. This is because it reduces the amount of reactive oxygen species made by human umbilical vein endothelial cells (HUVECs) and the constriction of blood vessels caused by thromboxane A2 [9]. Flavonoids and tannins have been linked to antioxidant activity [7] and vitamins content [10]. Due to their stability in gastric media, several of these phytochemicals might behave differently *in vivo*. To address this, the composition containing the extract may increase its activity.

The solvent concentration and kind have a substantial effect on the extraction of bioactive chemicals from plant materials. Different solvents have varying polarities and selectivity, which can impact the solubility and extraction efficiency of specific compounds. Specifically, acetone is effective at extracting proanthocyanidins and tannins, whereas ethanol is effective at extracting flavonoids and their glycosides, catechols, and tannins. On the other hand, methanol is often preferred for extracting phenolic acids and catechins. These observations align with the polarity of the solvents and their solubility characteristics. Higher solvent concentrations generally lead to increased extraction efficiency, but certain compounds, such as phenols or flavonoids, may exhibit different behaviors. Pure solvents without the addition of water may result in reduced extraction efficiency. Knowing how solvent concentration and type affect the extraction process is critical for appropriately analyzing the phenolic chemicals found in plant tissues [11].

The goal of this study is to see how different solvents, such as ethanol, methanol, acetone, and water, and different concentrations affect the way antioxidant phenolic compounds are extracted from lemongrass (*C. citratus*). Also, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical elimination assay will be used to measure how well the extracts work as antioxidants. Using a comprehensive single-factor experimental design, we hope to gain a deeper understanding of the extraction process and its effect on the antioxidant potential of lemongrass.

## 2. Materials and methods

### *2.1. Chemicals*

Montreal, Quebec-based Biochem, Chemopharma produced the Folin-Ciocalteu reagent. Disodium hydrogen phosphate ($Na_2HPO_4$), sodium carbonate ($Na_2CO_3$), ethanol, acetone, and methanol were provided by Prolabo (manufactured in CE). Quercetin and gallic acid manufactured in the United Kingdom by Biochem Chemopharma. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma-Aldrich (Steinheim, Germany).

### *2.2. Plant material*

The *Cymbopogon citratus* utilized in this investigation was gathered in June 2023 from a farm in Oued Souf (Algeria). *Cymbopogon citratus* was identified by Prof. Chehma A. (Ouargla University) and a voucher specimen (C-C-06) was deposited in the Laboratory of Process Engineering, Faculty of Applied Sciences, Kasdi Merbah University, Ouargla, 30000, Algeria.

### *2.3. Plant extraction*

Following plant transportation to the laboratory and before being merged and lyophilized in a lyophilizer (Alpha1-4 LDplus, Christ, Osterode, Germany), the plant was rinsed and desiccated. Before examination, freezing *C. citratus* samples were stored at 4 $^{o}$C. We extracted 15 mL of the extracting solvents from 500 mg of dried *Cymbopogon citratus* powder in a glass container. Mainly three extracting solvents were acetone, ethanol, and methanol with different concentrations from each solvent (20%, 40%, 60%, 80%, 100%). Different concentrations of water were used in a shaker bath to accomplish the extraction. After 20 minutes in a centrifuge at 5000 rpm (NF 200, Nuve, Turkey), the mixture was filtered using Watman filter paper. On three separate occasions, the extraction process was performed. Antioxidant activity was evaluated by measuring total phenolic content (TPC),

total flavonoid content (TFC), and DPPH radical-scavenging activity (DPPH RSA) in the filtered extracts.

### *2.4. Experimental methods*

*2.4.1. Quantitative determination of total polyphenol and flavonoids contents*

2.4.1.1. Total flavonoid compound determination. The amount of total flavonoid was calculated using the aluminum chloride complex-making method described by Santas *et al.* [12]. *Cymbopogon citratus* extract (1 mL) was mixed with $AlCl_3$ methanolic solution (2% w/v) (1 mL). The absorbance at 410 nm was then measured against a blank after the combination had been allowed to react at room temperature for 10 minutes. The total flavonoid content, expressed as mg of quercetin equivalent per 100 g of dry weight (mg QE/100 g DW), was calculated using a calibration curve. We took three separate readings of each parameter.

2.4.1.2. Total phenolic compound determination. The Folin-Ciocalteu method was used to determine the concentration of TPC in *C. citratus* extracts [13]. Folin-Ciocalteu's reagent (diluted 10 times with water) and sodium carbonate (7.5% (w/v)) were added to the sample volume (200 µL) in test tubes. After the tubes were mixed, a 30 minute incubation period was conducted at room temperature with the lights off. A Shimadzu UV mini1240 UV/VIS Spectrophotometer from Suzhou, Jiangsu, China was used to measure absorbance at 765 nm and compare it to a blank. Using a calibration curve, the total phenolics content was calculated as the gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW). We took three separate readings of each parameter.

*2.4.2. DPPH radical-scavenging activity (DPPH-RSA).* Antioxidants turned the violet stable radical DPPH into the yellow diphenyl picryl hydrazine (DPPH-H) in the DPPH experiment. To perform the test, an alcoholic DPPH solution is reduced in the presence of a hydrogen-donating antioxidant, resulting in the creation of the non-radical form DPPH-H [14]. Using Blois' formula, we determined that *C. citratus* extracts had a scavenging effect against DPPH radicals. To recap, 0.1 mL of each sample extract was combined with 0.9 mL of 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, and 0.12 mg/mL DPPH methanolic solutions. The absorbance at 517 nm was measured after being left at room temperature for 20 minutes. We took three separate readings of each parameter, and the inhibition (%) is calculated from the following equation:

$$Inhibition(\%) = \frac{A_0 - A_t}{A_0} \times 100$$

where $A_0$ = the absorbance of the DPPH methanolic solution and $A_t$ = the absorbance of the samples with DPPH solution, and the result was the percentage of radical-scavenging activity.

*2.4.3. Antibacterial and anti-fungal activity.* The technique of disc diffusion was employed to test the *in vitro* antibacterial efficacy of *Cymbopogon citratus* extracts on the following bacterial strains *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC6538), and *E. coli* (ATCC 25922). Additionally, two fungi were tested, *Candida albicans* (ATCC 14,053) and *S. cerevisiae* (ATCC 24657). One colony of bacteria was cultured overnight, and a sample was taken the next day and suspended in physiological water. Using a UV spectrometer (wavelength = 625 nm), the turbidity of the microbial suspensions was measured and set to 0.6 - 0.8 (in terms of optical density, or OD). Placing a lawn of bacteria on top of plates with Tryptic Soy Agar for bacteria and Sabouraud dextrose agar for the fungal sample was accomplished by dipping a sterile cotton swab into the resulting suspension. We next soaked sterile filter paper discs (diameter: 6 mm, Whatman paper No. 3) with 30 µL of each DMSO extract (6 mg/ mL). That way, the particles might come into contact with the agar and bacteria underneath. Both the bacterial and fungal plates were incubated for 24 hours at 37 $^oC$, while the yeast plates were incubated for 48 hours at 25 $^oC$. The sizes of the growth-inhibition zones were finally measured with the use of a zone inhibition reader (Fisher Lilly Antibiotic Zone Reader). Antibiotic discs like Gectapen® (40 mg/mL) served as positive controls, while 30 µL of distilled water served as a negative control. There were two sets of each experiment [15].

### *2.5. Statistical analysis*

The data presented herein represents the mean of three replicates along with their corresponding standard deviation (SD). A multivariate analysis of variance was employed to analyze the results, followed by mean comparisons conducted using Tukey's multiple range test with SPSS version 20.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). Significance was determined at $p < 0.05$. To elucidate the relationship between the chemical composition of plants and their antioxidant or other biological activities, linear correlation coefficients were calculated. Additionally, for exploratory data analysis, the results underwent multivariate analysis techniques, particularly principal components analysis (PCA). PCA was conducted using XLSTAT (version 2020.1, Addinsoft, Pearson edition, Waltman, MA, USA) to improve discrimination between the investigated parameters.

## 3. Results and discussion

### *3.1. Total phenolic and flavonoid content*

Phenolic components from *C. citratus* were extracted using different concentrations (20% to 100%; v/v) of ethanol, methanol, and acetone. The results demonstrated significant variations in phenolic extraction across all concentrations as seen in Table 1. The highest TPC yield was obtained with acetone solvent system followed by methanol, while ethanol yielded the lowest TPC (Figures 1-3). The highest TPC was obtained with 80% acetone (370.2 mg GAE/100 g), followed by 60% acetone (353.9 mg GAE/100 g); 40% acetone (333.4 mg GAE/100 g); and pure acetone (322.7 mg GAE/100 g) (Figure 1). Intermediate TPC values were compared favorably with the total phenolics content 644.4 mg/GAE g reported for lemongrass [16, 17].

**Table 1.** Total phenolic and flavonoid content of *Cymbopogon citratus* extracts

| Metabolites | Solvent concentration | Acetone | Ethanol | Methanol |
|---|---|---|---|---|
| Total phenolic content (mg GAE/ 100 g) | 100% | 322.7±0.43$^{d}$ | 250.4±0.11$^{j}$ | 287.9±0.41$^{i}$ |
| | 80% | 370.2±0.35$^{a}$ | 291.3±0.01$^{l}$ | 302.4±0.67$^{f}$ |
| | 60% | 353.9±0.49$^{b}$ | 300.1±0.24$^{f}$ | 307.4±0.92$^{e}$ |
| | 40% | 333.4±0.50$^{c}$ | 292.8±0.73$^{gh}$ | 292.3±0.47$^{h}$ |
| | 20% | 293.7±0.27$^{g}$ | 212.4±0.19$^{h}$ | 243.6±0.12$^{k}$ |
| Total flavonoids content (mg QE/ 100 g) | 100% | 32.7±0.65$^{g}$ | 39.8±0.32$^{e}$ | 43.9±0.57$^{d}$ |
| | 80% | 39.9±0.78$^{e}$ | 51.1±0.14$^{c}$ | 57.1±0.27$^{a}$ |
| | 60% | 35.6±0.57$^{f}$ | 58.7±0.18$^{a}$ | 55.2±0.65$^{b}$ |
| | 40% | 27.6±0.39$^{h}$ | 41.1±0.52$^{e}$ | 44.2±0.31$^{d}$ |
| | 20% | 16.8±0.80$^{i}$ | 27.1±0.55$^{h}$ | 31.1±0.90$^{g}$ |

Values are mean ± standard deviation of triplicate experiments.
Means with different superscript letters indicate significant differences ($p < 0.05$) according to Tukey test.

In a similar way, extracting total flavonoids content from *C. citratus* demonstrated that methanol and ethanol were the solvent of choice in extracting flavonoids from *C. citratus* as shown in Table 1. TFC extraction efficiency was greater with 60% ethanol (58.7 mg QE/100 g), followed by 80% methanol extraction (57.1 mg QE/100 g), 60% methanol (55.2 mg QE/100 g), and 80% ethanol (51.1 mg QE/100 g) (Figures S1-3).

The outcomes of Tukey's test underscore significant disparities among solvents regarding their efficacy in extracting polyphenols. Notably, 80% acetone emerged as the most effective solvent, trailed by 60%, 40%, and 100% acetone, in descending order of efficiency. This analysis unequivocally designates acetone as the optimal solvent for polyphenol extraction. Conversely, the weakest solvents for polyphenol extraction were determined to be 20% methanol and 20% ethanol, based on the statistically significant differences revealed by Tukey's test.

In contrast, distinct variations in solvent effectiveness were observed concerning flavonoid extraction. Specifically, 60% ethanol exhibited the highest efficacy for extracting flavonoids, followed closely by 60% methanol. These findings emphasize the pivotal role of solvent type and concentration in extracting phytochemical content, as elucidated by Tukey's test. The discernible variation in the effect of solvents underscores the critical importance of solvent selection and concentration in optimizing phytochemical extraction processes.

### *3.2. DPPH radical-scavenging activity (DPPH-RSA)*

Table 2 shows the results of an analysis of the antioxidant activity of *Cymbopogon citratus* extracts, determined by their capacity to scavenge DPPH free radicals. Reduced absorbance and a shift in color from violet to yellow occur when an antioxidant scavenges the DPPH radical, converting it into the more stable DPPH-H molecule [18]. The $IC_{50}$ is the amount of an antioxidant-containing material needed to scavenge 50% of the initial DPPH radicals. Higher antioxidant activity is implied by a substance's greater ability to scavenge DPPH and a lower $IC_{50}$ value [19].

**Table 2.** Free radical-scavenging ability of *Cymbopogon citratus* extracts measured by DPPH test in terms of $IC_{50}$ value (µg/mL of extract)

| Solvent concen-tration | $IC_{50}$ value (µg/mL of extract) | | |
|---|---|---|---|
| | Acetone | Ethanol | Methanol |
| **100%** | 21.74±0.29$^{b}$ | 24.36±0.22$^{efg}$ | 24.65±0.90$^{fg}$ |
| **80%** | 21.16±0.28$^{b}$ | 22.19±0.75$^{bcd}$ | 21.96±0.21$^{bc}$ |
| **60%** | 23.13±0.57$^{cde}$ | 19.22±0.35$^{a}$ | 21.65±0.44$^{b}$ |
| **40%** | 22.06±0.36$^{bc}$ | 22.15±0.30$^{bc}$ | 22.25±0.25$^{bcd}$ |
| **20%** | 23.48±0.36$^{def}$ | 24.87±0.39$^{g}$ | 25.35±0.24$^{g}$ |

Values are mean ± standard deviation of triplicate experiments.
Values with different superscripts letter indicate significant differences ($p < 0.05$) according to Tukey test.

The highest DPPH-RSA $IC_{50}$ value reported was 19.22 for ethanolic extract at 60% concentration which is correlated mainly to its greatest total flavonoid content (58.7 mg QE/100 g) in addition to 300.1 mg GAE/100 g) as a TPC (Figure 6). Additionally, acetone extract also had a great antioxidant capacity as the radical scavenging activity of DPPH (DPPH-RSA) $IC_{50}$ for acetone (80%) was 21.16 (Figure 4), which was related to its greatest total phenolic content (370.2 mg GAE/100 g). While for methanol at 60% concentration, $IC_{50}$ was 21.65 which was not substantially different from acetone (Figure 5). Our findings agree with those of González *et al.* [20], who demonstrated that extracting phenols from banana peels using 50% acetone was more successful, resulting in extracts with increased antioxidant activity. Thus, acetone was selected as the optimal solvent for improving extraction conditions without increasing TPC production or reducing antioxidant efficacy (DPPH-RAS). The kind of solvent (polarity) has a significant impact on the solubility of phenolic compounds, the degree of polymerization, food interactions, and the production of insoluble compounds [21]. Acetone concentration had a significant influence on the DPPH-RSA of *Cymbopogon citratus* extracts. The greatest values for DPPH-RSA were achieved at 80% acetone, with 97.4% and 370.2 mg GAE/100 g, respectively. The best acetone gradient for the subsequent steps was determined to be 80% (Figure 1).

Methanol and ethanol concentrations exhibited a substantial effect on the DPPH-RSA of *C. citratus* extracts. For DPPH-RSA, the greatest values were found at 60% methanol and 60% ethanol. As a result, the optimal acetone gradient for the following steps was decided to be 60% methanol and ethanol. Organic solvents in water mixtures contribute to the formation of a very polar medium, which facilitates polyphenol extraction. However, phenol identification and quantification might be hindered by the presence of contaminants in an extract made using water as the only solvent, such as organic acids, sugars, and soluble proteins (Figures 2 and 3) [22].

For the extraction of phenols from various natural sources, acetone-water combinations (40-70% v/v) have been characterized as one of the most successful solvents, similar to the present study [23]. The most

efficient solvent for extracting phenol from date seeds was determined to be 50% acetone.

Meneses *et al*. [24]. were the ones who discovered that the most effective solvent for removing the phenolic antioxidant components from beer beans was determined to be 60% acetone.

The water mixture has the ability to degrade polyphenol and protein complexes. This explains why this solvent is so effective in extracting phenolic chemicals. Downey and Hanlin [25] found that acetone mixtures containing 50% to 80% acetone remove the most condensed tannins from grape skins. Kalithrasas and others [26] discovered that the optimum solvent for the extraction of grape seed phenols, particularly proanthocyanidins was 70% acetone.

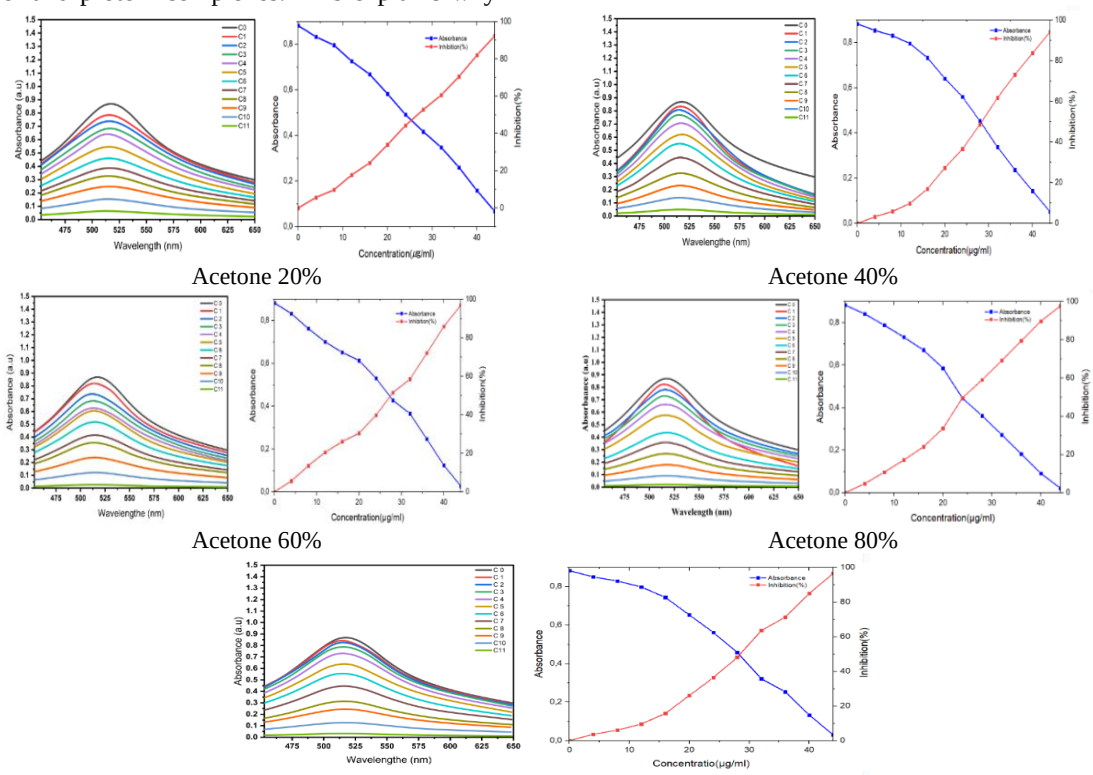


**Figure 1.** Antioxidant activity from acetone extracts of *Cymbopogon citratus*

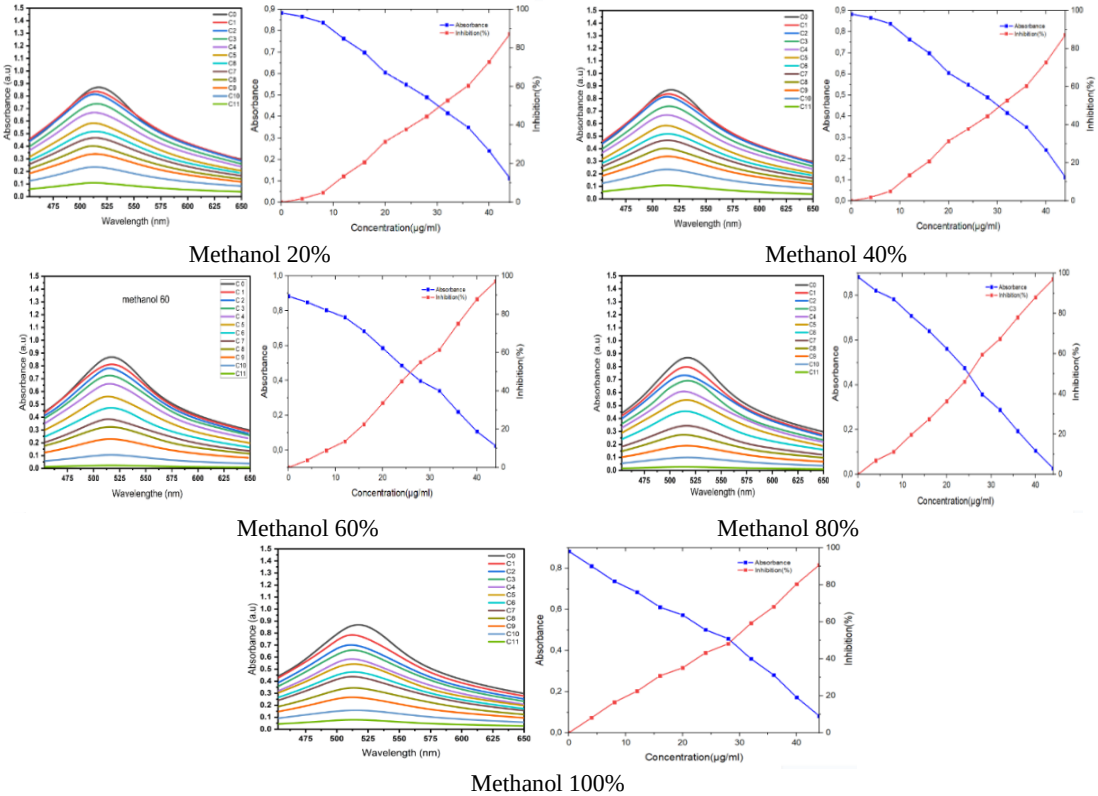


**Figure 2.** Antioxidant activity from methanol extracts of *Cymbopogon citratus*

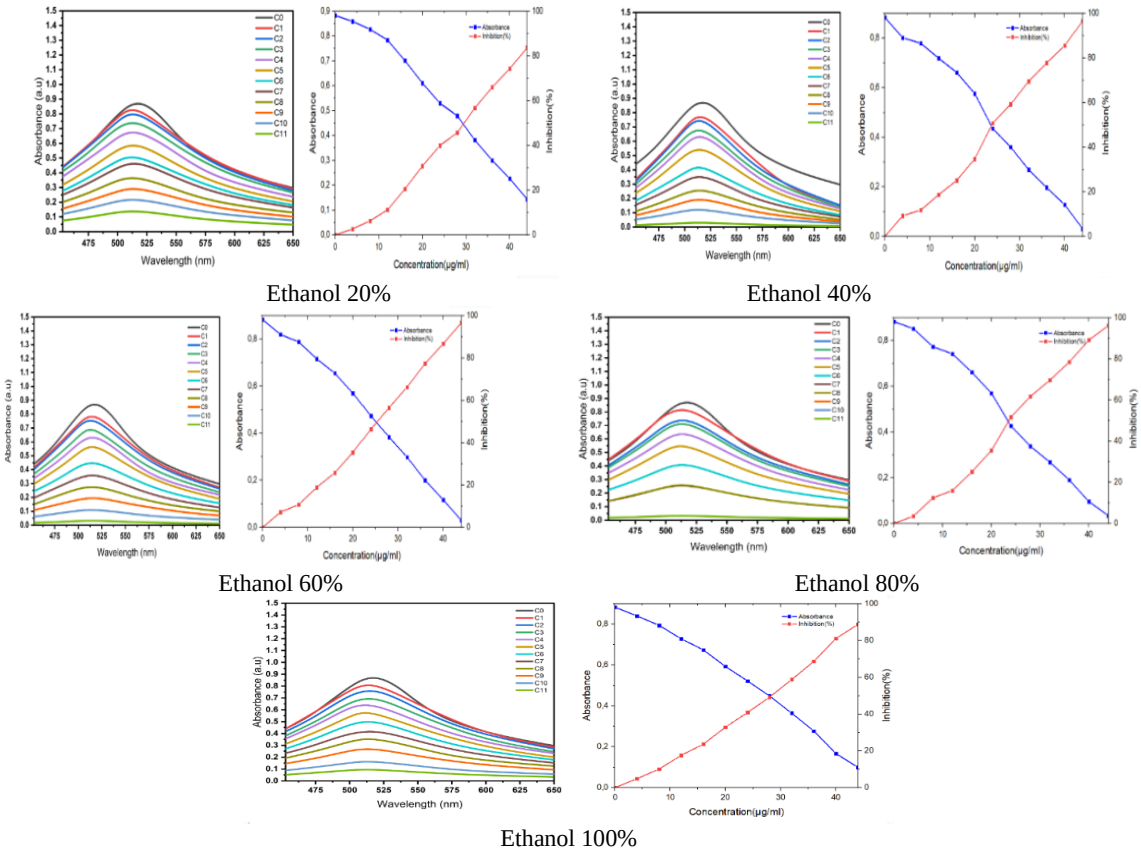


**Figure 3.** Antioxidant activity from ethanol extracts of *Cymbopogon citratus*

### *3.3. Antibacterial activity*

Extracts of *C. citratus* were tested for their antibacterial efficacy against a variety of microorganisms, Gram (+) bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and Gram (−) bacteria such as *Pseudomonas aeruginosa* and *Eschericia coli* as listed in Table S1 and illustrated in Figures 6-9 . The extracts were prepared using various solvents and concentrations (acetone, methanol, and ethanol) at different concentrations (1, 5, and 10 µg/mL). The inhibitory action results in the appearance of a transparent halo (inhibition zone) around the paper disc that has been impregnated with extracts under study. In the current study the inhibition zone diameters listed in Table 3 were calculated after subtracting 6 mm which represents the diameter of the sterile filter paper discs (diameter: 6 mm, Whatman paper No. 3) (Figure 4).

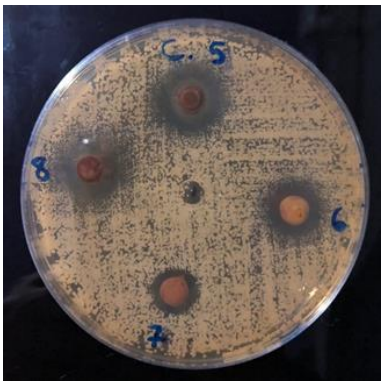

**Figure 4.** Inhibition zones of *Cymbopogon citratus* extracts representing its antibacterial activity

Several critical factors affect the antibacterial action. The differences in the antibacterial activity of *C. citratus* against these two different types of bacteria may be related to changes in the structure and chemical composition of the cell membrane, and especially the nature of the cell wall. The periplasmic region, which acts as a barrier between the outer and inner membranes and allows entry of inhibitory compounds, is easily solubilized by the thin outer membrane [27] (Figure 5).

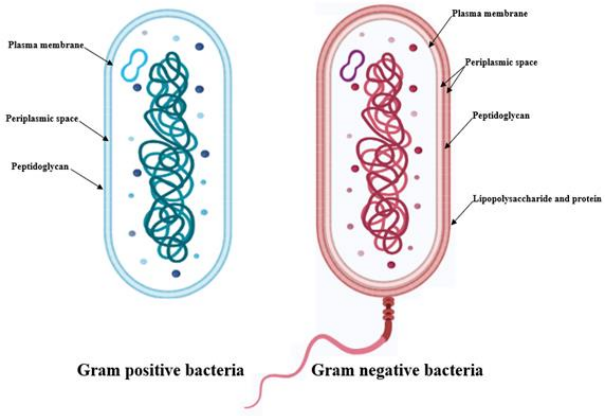


**Figure 5.** Comparative schematic representation of Gram-positive and Gram-negative bacteria

From the results, for *E. coli*, the acetone 100% extract showed moderate inhibition with zone (IZ) diameters of 7, 11, and 13.5 mm, while acetone 80%, 60%, 40%, and 20% extracts exhibited increasing IZ of 10, 13, and 16 mm, 9, 12, and 14 mm, 7, 9, and 12 mm, and 7, 8, and 10 mm, respectively. The methanol and ethanol extracts demonstrated relatively higher activity

against *E. coli*, with inhibition zones ranging from 6 to 16 mm as seen in Figure 8. Against *Pseudomonas aeruginosa*, the acetone 100%, 80%, 60%, and 40% extracts displayed moderate activity with inhibition zones of 12, 13, and 15 mm, 13, 14, and 17 mm, 11, 12, and 14 mm, and 10, 11, and 13 mm, respectively, while the acetone 20% extract showed slightly lower activity with zones of 8, 9, and 11 mm. This study has been reported to have antimicrobial properties (Figure 6) [28] .

The methanol and ethanol extracts exhibited significant inhibition zones ranging from 6 to 16 mm against *Pseudomonas aeruginosa*. Concerning *Bacillus subtilis*, the acetone 100%, 80%, 60%, and 40% extracts exhibited moderate activity with inhibition zones of 6, 8, and 9 mm; 8, 9, and 11 mm; 8, 9, and 10 mm; and 6, 7, and 8 mm, respectively, while the acetone 20% extract showed the lowest activity with zones of 4, 4, and 6mm. Significant inhibition zones ranging from 5 to 16 mm were seen against *Bacillus subtilis* in the methanol and ethanol extracts (Figure 7).

Overall, acetone and methanol extracts demonstrated the highest antibacterial activity compared to ethanol extracts, 80% acetone, and 100% methanol showed the most potent activity against the tested bacterial strains. For *Streptococcus aureus*, as seen in Figure 8, the 20% acetone extract exhibited moderate activity with IZ of 8, 9, and 10 mm. While the inhibition zones in the methanol extracts varied, the 100% extract displayed 8, 9, and 11 mm, the 80% extract displayed 10, 11, and 12 mm, the 60% extract had 8, 9, and 10 mm, and the 40% extract exhibited 7, 8, and 9 mm. The inhibition zones in the 20% methanol extract were 6, 7, and 8 mm. The zones of inhibition in the extracts of ethanol ranged from 6 to 14 mm, with the 100% extract having 10, 13, and 14, the 80% extract having 10, 13, and 15, the 60% extract having 8, 11, and 11, the 40% extract having 7, 9, and 9, and the 20% extract having 4, 6, and 7. Overall, acetone and methanol extracts have better antibacterial activity against *Streptococcus aureus* than ethanol extracts. The acetone 80% and methanol 80% extracts revealed much better antibacterial activity, with inhibition zones ranging from 10 to 15 mm. Bactericidal effects were likewise seen in the 100% ethanol and 100% methanol extracts, with inhibition zones ranging from 10 to 14 mm. The inhibition zones for the acetone 100% and methanol 40% extracts ranged from 7 to 11 mm, indicating modest antibacterial activity (Figure 9). Circles of inhibition measured between 4 and 9 mm in diameter for the acetone 20% and ethanol 20% extracts, indicating the weakest antibacterial activity. Similar findings, in which inhibition was nil over a range of extract concentrations, were reported in earlier research [29].

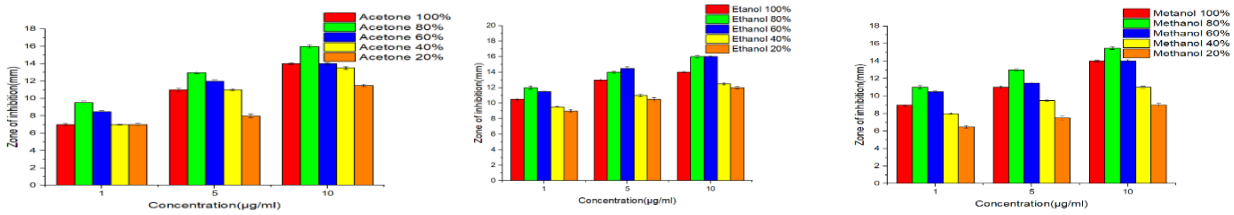


**Figure 6.** Antibacterial activity of *Cymbopogon citratus* extracts against *E. coli*

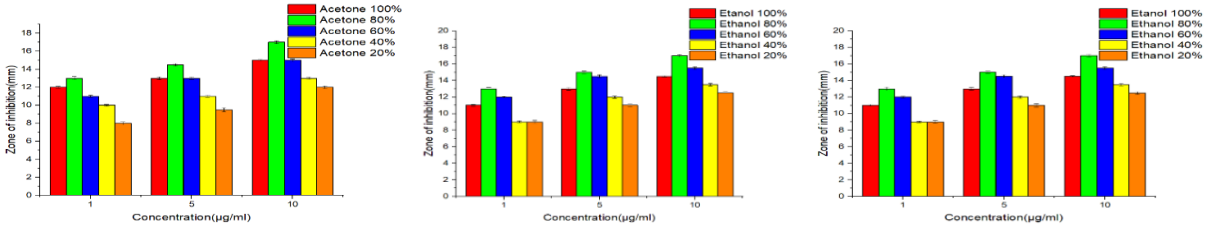


**Figure 7.** Antibacterial activity of *Cymbopogon citratus* extracts against *Pseudomonas aeruginosa*

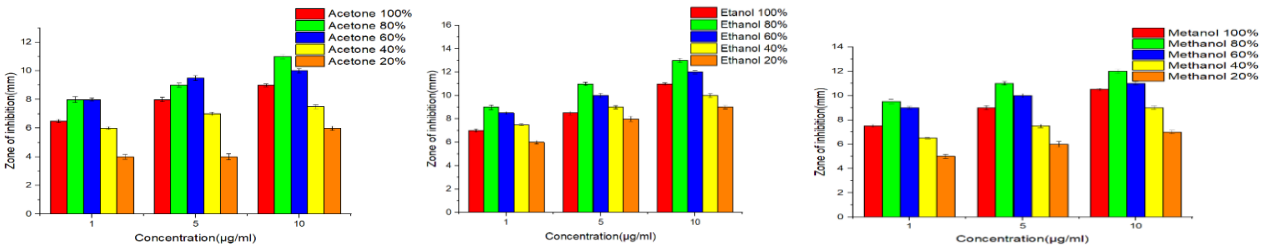


**Figure 8.** Antibacterial activity of *Cymbopogon citratus* extracts against *Bacillus subtilis*

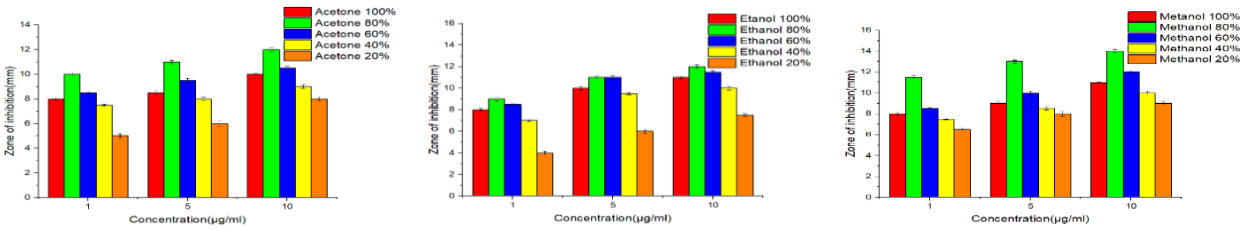


**Figure 9.** Antibacterial activity of *Cymbopogon citratus* extracts against *Streptococcus aureus*

### *3.4. Anti-fungal activity*

Two different types of molds were used to investigate *C. citratus* extracts' antifungal efficacy, *Candida albicans* (*C. albicans*) and *Saccharomyces cerevisiae* (*S. cerevisiae*), in various solvents (acetone, methanol, and ethanol) at doses of 1, 5, and 10 µg as showed in Table S2The acetone extracts for *C. albicans* showed IZ diameters ranging from 6 to 14 mm, with 100% extract showing 11, 12, and 14 mm; 80% extract showing 12, 14, and 15 mm; 60% extract showing 10, 11, and 12 mm; 40% extract showing 8, 8, and 10 mm; and 20% extract showing 6, 7, and 7 mm. Among the methanol extracts, inhibition zone diameters ranged from 6 to 12 mm, with the 100% methanol extract displaying 9, 11, and 12 mm, and the 80% methanol extract showing 12, 14, and 15 mm at concentrations of 1, 5, and 10 µg/mL, respectively (Figure 10).

Similarly, the inhibition zone diameters for the ethanol extracts ranged from 7 to 13 mm, with 100% ethanol extract showing 8, 11, and 12 mm at concentrations of 1, 5, and 10 g and 80% ethanol extract showing 11, 13, and 15 mm at those same concentrations.

For *S. cerevisiae*, the acetone extracts exhibited inhibition zone diameters ranging from 9 to 18 mm, with the 100% extract showing 12, 14, and 17 mm; 80% extract exhibiting 13, 16, and 18 mm; 60% extract having 13, 14, and 15 mm; 40% extract demonstrating 12, 13, and 15 mm; and 20% extract displaying 9, 10, and 11 mm. Among the methanol extracts, inhibition zone diameters ranged from 8 to 14 mm, with 100% methanol extract exhibiting 9, 11, and 13 mm; and 80% methanol extract showing 12, 13, and 15 mm at concentrations of 1, 5, and 10 µg, respectively (Figure 11). Similar to this, inhibition zones for ethanol extracts measured between 8 and 15 mm in diameter at 100% and 80% ethanol concentrations, respectively, indicating 8, 11, and 13 mm and 11, 14, and 15 mm for the inhibition zones. When tested against *Candida albicans* and *Saccharomyces cerevisiae*, ethanol extracts usually showed less antifungal efficacy than acetone and methanol extracts (respectively 80% and 80%).

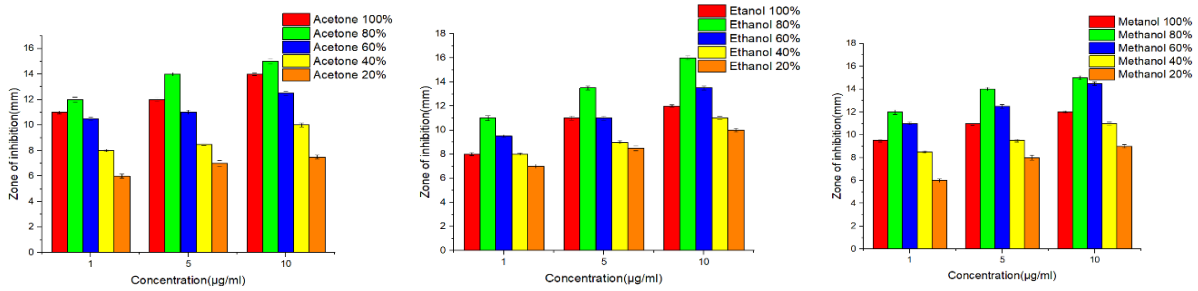


**Figure 10.** Antifungal activity of *Cymbopogon citratus* extract against *C. albicans*

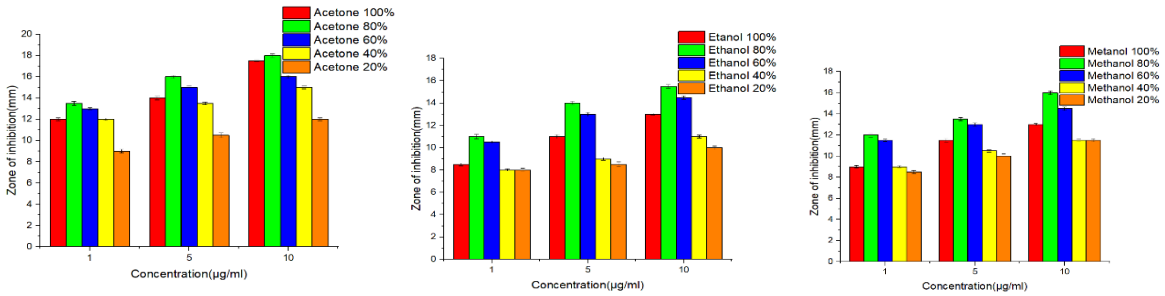


**Figure 11.** Antifungal activity of *Cymbopogon citratus* extract against *S. cerevisiae*

### *3.5. Correlation between solvents extract and phytochemical content, antioxidant, antibacterial, and antifungal activity*

PCA was employed to delve deeper into the relationship between the overall content of various chemical components across 15 extracts and their respective antioxidant, antibacterial, and antifungal activities, as depicted in Figure 12 and Table 3. The investigation aimed to elucidate the interplay between the two primary constituents of the 15 plant extracts, denoted as Total Phenolic Content and Total Flavonoid Content, in

relation to antioxidant, antibacterial, and antifungal activities.

According to the results of the Tukey test presented in Table 1, it is evident that solvents consisting of 80% acetone and 60% acetone yield the highest concentration of polyphenols, while solvents comprising 60% acetone and 80% methanol extract the highest concentration of flavonoids.

Statistical analysis revealed a negative correlation between chemical compounds and the results of the DPPH test. This is attributed to the greater effectiveness of antioxidant activity observed at lower $IC_{50}$ concentrations.

Table 5 highlights a robust correlation between TPC concentration and the DPPH test, evidenced by a coefficient of -0.62231, signifying a substantial connection. Similarly, TFC concentration exhibits a strong correlation with the DPPH test, with a coefficient of -0.54731.

Furthermore, Table 5 demonstrates a significant correlation between chemical compounds and antibacterial/antifungal activities. Flavonoid concentration exhibits a notably strong correlation with antibacterial activity against various strains, including *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*, as indicated by correlation coefficients ranging from 0.69184 to 0.88101. Conversely, the correlation between polyphenol concentration and antibacterial activity ranges from weak to moderate.

The correlation of antifungal activity with polyphenols and flavonoids varies, with *Candida albicans* showing a stronger correlation with flavonoids compared to polyphenols, while *S. cerevisiae* demonstrates a notable correlation with polyphenols.

Figure 15 depicts a robust correlation between polyphenol concentration and inhibition of *S. cerevisiae*, particularly when acetone is used as the extraction solvent at concentrations of 100%, 80%, and 60%. Meanwhile, inhibition of *Candida albicans*, *S. aureus*, and *P. aeruginosa* shows a strong association with methanol and ethanol solvents at concentrations of 60% and 80%. The association between *E. coli* and *B. subtilis* and flavonoid concentration with these last two was very strong.

Notably, the DPPH test exhibits significant efficacy with methanol and ethanol solvents at a concentration of 20%.

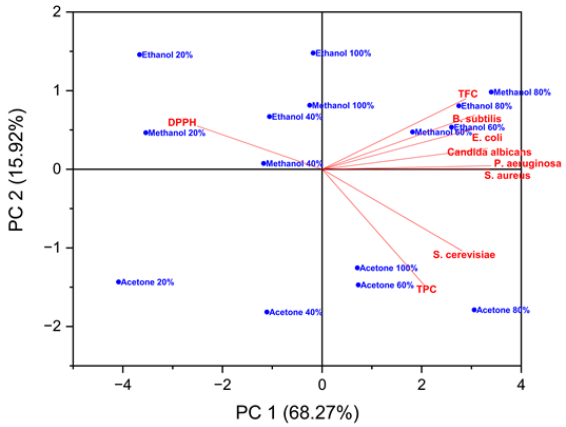


**Figure 12.** Biplot principal component analysis of TPC, TFC and antioxidant, antibacterial, antifungal activity of plant extracts. The percentages PC 1 and PC 2 represent the largest and second largest variance in the data on the X-axis and Y-axis, respectively

**Table 3**. Correlation matrix between chemical content and antioxidant, antibacterial, antifungal activity of plant extracts

| | TPC | TFC | DPPH | *E. coli* | *P. aeruginosa* | *B. subtilis* | *S. aureus* | *Candida albicans* | *S. cerevisiae* |
|---|---|---|---|---|---|---|---|---|---|
| **TPC** | 1 | 0,13716 | -0,62231 | 0,23478 | 0,45666 | 0,27229 | 0,5666 | 0,38976 | 0,82409 |
| **TFC** | 0,13716 | 1 | -0,54731 | 0,74971 | 0,69184 | 0,88101 | 0,75939 | 0,72578 | 0,31603 |
| **DPPH** | -0,62231 | -0,54731 | 1 | -0,56634 | -0,54499 | -0,49153 | -0,52476 | -0,46744 | -0,56984 |
| ***E. coli*** | 0,23478 | 0,74971 | -0,56634 | 1 | 0,82904 | 0,74886 | 0,74628 | 0,77687 | 0,53137 |
| ***P. aeruginosa*** | 0,45666 | 0,69184 | -0,54499 | 0,82904 | 1 | 0,75216 | 0,86769 | 0,94773 | 0,79891 |
| ***B. subtilis*** | 0,27229 | 0,88101 | -0,49153 | 0,74886 | 0,75216 | 1 | 0,79035 | 0,81294 | 0,44231 |
| ***S. aureus*** | 0,5666 | 0,75939 | -0,52476 | 0,74628 | 0,86769 | 0,79035 | 1 | 0,88605 | 0,7332 |
| ***Candida albicans*** | 0,38976 | 0,72578 | -0,46744 | 0,77687 | 0,94773 | 0,81294 | 0,88605 | 1 | 0,68398 |
| ***S. cerevisiae*** | 0,82409 | 0,31603 | -0,56984 | 0,53137 | 0,79891 | 0,44231 | 0,7332 | 0,68398 | 1 |

## 4. Conclusions

In conclusion, it should be noted that *Cymbopogon citratus* (DC. ex Nees) Stapf. (Poaceae) is a highly valuable plant with a variety of applications, making it commercially significant worldwide. This work examined the effects of varying solvent types and concentrations on the extraction process, with a particular focus on the analysis of phenolic compounds in *C. citratus*. Total phenolic content and total flavonoid content were extracted from *C. citratus* extracts using three different solvents: acetone, ethanol, and methanol, in concentrations ranging from 20% to 100%. Quantitative analysis revealed that the 80% acetone extract contained the highest concentration of polyphenolic compounds (370.2 mg GAE/100 g), followed by the 60% acetone extract (353.9 mg GAE/100 g). While 60% ethanol extract exhibited the highest total flavonoid concentration (58.7 mg QE/100 g).

Extracts of different polarities from *C. citratus* demonstrated effective antibacterial and antifungal activity. According to the findings, the acetone 60% extract was most efficient against *Pseudomonas aeruginosa*, but the acetone 80% extract was more active against *Saccharomyces cerevisiae*. For antifungal activity, the extracts with 80% acetone and 80% methanol showed the best results against

*Saccharomyces cerevisiae* and *Candida albicans*, respectively.

Regarding antioxidant capacity, the ethanol extract at 60% concentration displayed the highest DPPH-RSA $IC_{50}$ value, which correlated with its significant total flavonoid content (58.7 mg QE/100 g) and total phenolic content (300.1 mg GAE/100 g). The results of this study provide additional insight into the phenolic content and biological functions of *C. citratus*, providing the basis for future investigation and application of its advantageous characteristics. Therefore, further studies should be undertaken to elucidate phytochemicals and their pharmacological mechanisms.

## Conflicts of interest

The authors declare no conflict of interest.

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