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*Original Article*

# Antibacterial Effect of Aqueous Extract of Theobroma Cacao against Gram-Negative and Gram-Positive Bacteria: An *in Vitro* Study

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**ABSTRACT**

*Theobroma* Cacao has a wide range against a range of diseases and an antimicrobial activity toward various pathogenic microorganisms which estimated of medicinal properties for the treatment of many infectious diseases. The aim of the research is to clarify the inhibitory action of *Theobroma* Cacao extract against human pathogenic bacteria also show the mechanism action of extract against biofilm formation and adherence of bacteria. The antimicrobial activity of the aqueous extract was tested based on agar-well diffusion assay and agar-disc diffusion, and this activity was compared with the antibiotic and measured which is more efficient. Furthermore, biofilm formation and adherence tests were done. *Theobroma cacao* (30%) was appeared in this research as high efficient against the wide spectrum of clinic isolates gram-negative and gram-positive bacteria (*K.pneumonia, P.merabilis, P.vulgaris, S.typhimurum, S.typhi,, Staph. aureus, Staph.epidermidis, Staph.saprophytics and Strep.Pyogenes*) which indicates that it is more effective than commercially available antibiotic in addition to a high inhibition against adherents and biofilm formation.

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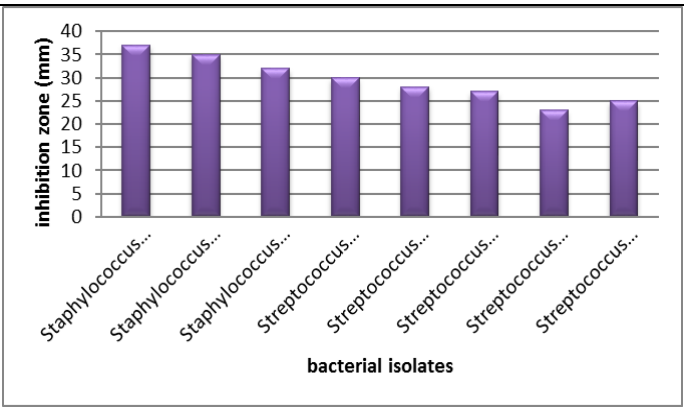
**GRAPHICAL ABSTRACT**




## Introduction

Plants have been a decent source of beneficial agents for thousands of years; the imposing quantities of current drugs used for treating human diseases are derived from natural sources [1]. Some of these plants have antioxidant, antibacterial, and anti-inflammation potentials like *Theobroma cacao*, popularly known as Cocoa, is a tree belonging to the family *Malvaceae which* comprises different chemical compounds like a high concentration of bioactive compounds, including polyphenols, tocopherols, flavonoids, including catechin, epicatechin, and procyanidins [2].

Some studies have been conventionally used for their anti-oxidant, immune-modulatory, and anti-microbial activities [3-5]. There were rare studies for *Theobroma cacao* estimating anti-microbial activity in addition to adherence and anti-biofilm activity in relation to pathogenic bacteria have been testified [6, 7].

The studied plant extracts of *Theobroma cacao* indicated the antimicrobial activity in addition to adherence inhibition and biofilm formation of some clinical isolates which assumed the significance of emerging new plans for controlling the growth of biofilm because of its pathogenic feature.

These results may signify the leading of numerous steps towards the expansion of novel antimicrobial, anti-biofilm, and anti-adherence drugs by extracts of *Theobroma cacao* used in old-style.

## Materials and Methods

For preparation of the aquatic extract from *Theobroma cacao*, the related aquatic extracts concentration (30%) and ciprofloxacin were obtained according to [8].

### *Bacterial and isolates*

19 bacterial isolates (clinical specimens) were used in this work. Bacterial isolates are presented in the following table. Bacterial isolates were activated in three continual times on nutrient agar, and then they were stored at 4 °C as nutrient agar slant. The isolates documentation was established by various biochemical tests [9]. Gram-negative and gram positive bacteria are listed in Table 1.

Antimicrobial activity test by agar-well diffusion assay *(In vitro) was d*epended on Hindi and Chabuck [10].

### *Antibacterial activity assay*

According to Forbes (2007) [9], the antimicrobial activity was detected by agar-disc diffusion for antibiotic (The tests were performed in triplicates).

### *Biofilm Formation Assay*

Semi-quantitative microtiter plate test or tissue culture plate method assay (TCP) designated by Christensen *et al*. (1985) [12] was expected as the best standard method for detecting biofilm formation (for only gram-negative bacteria) which presented in Table 2.

**Table 1:** Gram-positive and gram-negative bacteria

| Gram- positive bacteria | Gram- negative bacteria |
|---|---|
| *Staphylococcus aureus* | *Salmonella typhi* |
| *Staphylococcus epidermidis* | *Salmonella typhimurum* |
| *Staphylococcus saprophyticus* | *Pseudomonas aeroginosa* |
| *Streptococcus pyogenes* | *Pseudomonas flourscences* |
| *Streptococcus pneumonia* | *Proteus vulgaris* |
| *Streptococcus mutanus* | *Proteus merabilis* |
| *Streptococcus feacalis* | *Klebsiella pneumonia* |
| *Streptococcus aglagtia* | *Enterobacter aerugenes* |
| | *Acinetobacter* |
| | *Escherichia coli* |
| | *Serratia spp.* |

**Table 2:** adherence and biofilm formation by method of TCP [11]

| OD mean value at 630 nm | Adherence | Biofilm formation |
|---|---|---|
| 0.120> | Non | Non |
| 0.240-0.120 | Moderately | Moderate |
| >0.240 | Strong | High |

## Results and Discussion

After the emergence of many difficult challenges left by the excessive use of antibiotics, our ancestors have used the extracts since the ancient times, and scientists have used them to clarify the mechanics of these extracts.

Researchers found the alternatives to antibiotics with a high efficacy and low toxicity to human, in addition to the cheapest price compared with antibiotics. Plant extracts have been used since the ancient times, whether in the form of aquatic or alcoholic extracts. In the present work, 19 bacterial isolates (clinical specimens) were used in the work. Bacterial isolates are indicated in Table 1 which established by various biochemical tests. *Theobroma cacao* (30%) was appeared in this research as high efficient against wide spectrum of clinic isolates (gram-negative and gram-positive bacteria) that represented the high inhibition zone of *K.pneumonia, P.merabilis, P.vulgaris, S.typhimurum, and S.typhi,* which ranged 33-30 mm, while the inhibition zone of gram-positive (*Staph.aureus, Staph.epidermidis, Staph.saprophytics, and Strep.Pyogenes*) ranged at 37-30 mm. The extract appeared high potential against these clinical isolates gram-negative and gram-positive bacteria (Figures 1 and 2).

When compared Figure 1, 2, 3, and 4, it was noted that cocoa was more efficient than the antibiotic ciprofloxacin (positive control used ciprofloxacin suspension). When gram-positive and gram-negative, we noted that *Theobroma cacao* is more efficient against gram-positive bacteria. This efficiency of *Theobroma cacao* related to chemical compounds mentioned by researchers (7) included the great concentration of bio-active composites, polyphenols, tocopherols, flavonoids, catechin, epicatechin, and procyanidins, and also it has chemical structure (tricyclic) of the flavonoids regulates acting as antioxidant effects that hunt reactive oxygen species, chelate $Fe^{2+}$ and $Cu^{+}$, inhibit enzymes, and upregulate antioxidant defenses. Other researches approved that *Theobroma cacao* was more efficient against some pathogenic bacteria (*the* inhibition zone of pathogens *Serratia marcescens, Staphylococcus aureus, Salmonella sp., and Shigella* dysenteriae) [13]. Likewise, *Theobroma s*howed a greater zone of inhibition against some pathogenic bacteria like *S.mutans, L.acidophilus*, and *E.faecalis*, whereas almost a neutral effect was observed for *C.albicans*. which was statistically significant, as compared with the other two toothpastes [14].

Table 2 demonstrates the efficiency of an aquatic extract for powder *Theobroma cacao* (30%) since inhibited the one of important virulence factors

of bacteria which pointed out to the high efficiency of inhibition adherence in 5 isolates of gram-negative bacteria including (*S.typhi, S.typhimurum, Acentobacter spp., E.coli, Serratia spp*.), while the 6 isolates (*Pseudo.flourscenes, Proteus vulgaris, Proteus merabilis, K.pnemoniae, Enterbacter spp., and Pseudo. aerugenes.*) appeared that moderate activity. Bacterial adhesion to the biomaterial surfaces is the essential step in the biofilm formation and pathogenesis of these infections.

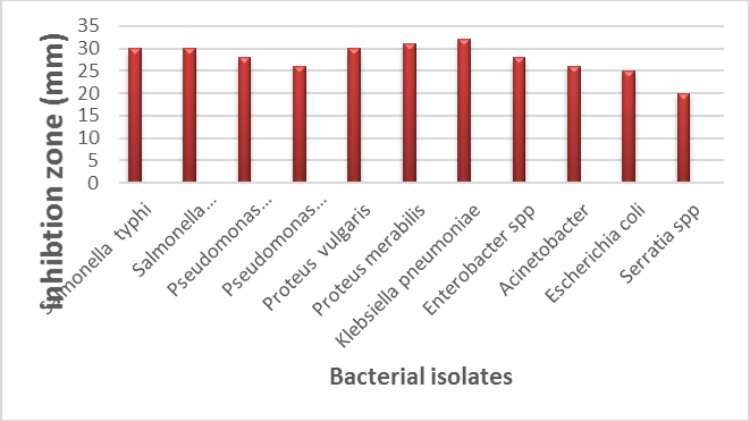



**Figure 1:** Antibacterial effect of *Theobroma cacao* against gram-negative bacteria by agar-well method

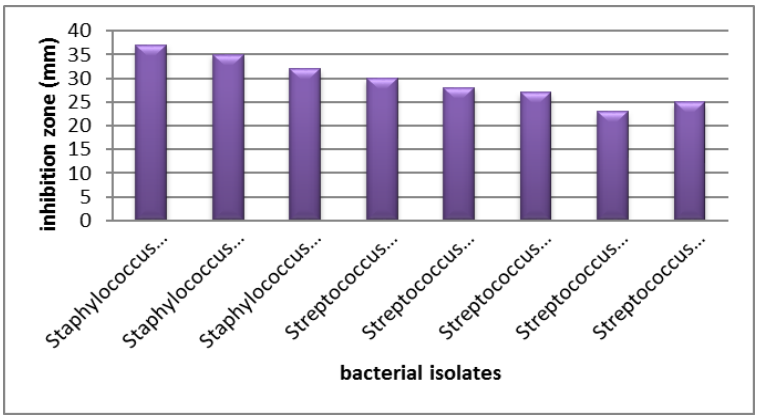



**Figure 2:** Antibacterial effect of Theobroma cacao against gram-positive bacteria by agar-well method

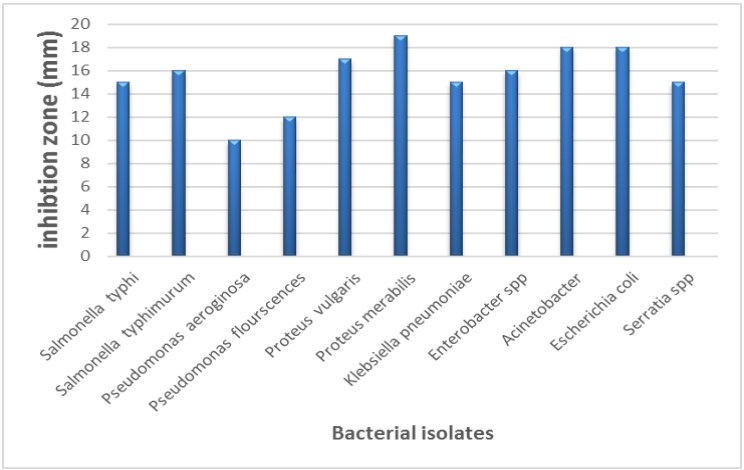



**Figure 3:** Antibacterial effect of *Ciprofloxacin* against gram-negative bacteria by agar-well method

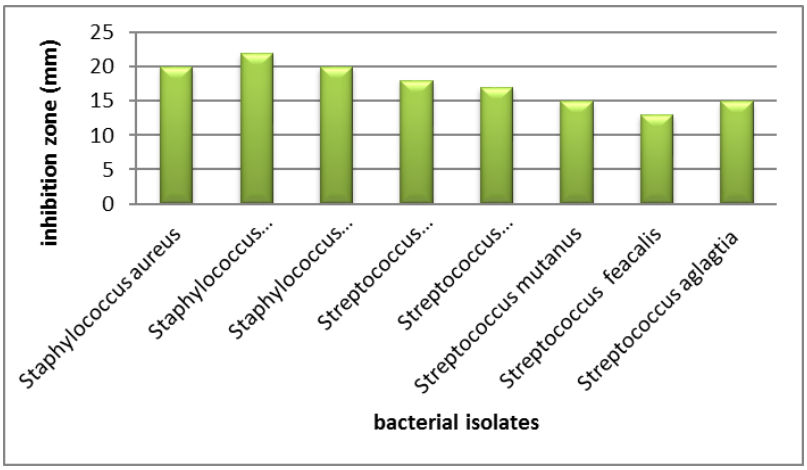



**Figure 4:** Antibacterial effect of *Ciprofloxacin* against gram-positive bacteria by agar-well method

**Table 3:** Anti-adherence activity of aquatic extracts of *Theobroma cacao* against gram-negative bacteria

| Gram negative bacteria | *Theobroma cacao* |
|---|---|
| *Salmonella typhi* | High |
| *Salmonellatyphimurum* | High |
| *Acinetobacter* | High |
| *Escherichia coli* | High |
| *Serratia spp.* | High |
| *Pseudomonas aeroginosa* | Moderate |

Bacterial biofilm-consider as infections constitute of wholly bacterial and prolonged infections in animals and humans [15, 16], while Table 3 includes the high mechanism of *Theobroma cacao* against the prevented form action of biofilm for the pathogenic bacteria, which can note the *Theobroma cacao* with a high action against S.*typhi, S.typhimurum, Acentobacter spp., E.coli, Serratia spp.* Whereas, the rest of pathogenic bacteria illustrated a moderate activity against *theobroma cacao* (*Pseudo.aeroginosa, Pseudo.flourscences, Proteus vulgaris, K.pneumoniae, Enterobacter aerugenes Acintobacter, E. coli,* and *Serratia spp.*). The results of this study appeared that when affected on cell adherence impacting the biofilm formation which appeared clear in this study.

The weakness of inactivation of biofilm formation may be associated to the cell adherence, and thus the plant extract showed that clarity affected on the biofilms elimination and/or inactivation pointed by Romero *et al.* [17].

The extracts from these medicinal plants may impact on many virulence factors like biofilm formation by destructive bacterial membrane organizations [18], constraining peptidoglycan creation, and/or modulating quorum sensing [19].

These facts may be related to have some chemical compounds like flavonoids that utilize anti-biofilm special effects via quorum sensing inhibition [20-30], In this research, *Theobroma cacao* extracts presented biofilm inhibition with flavonoids [2], and intrinsically, these complexes may impact on the biofilm inhibition experimental. The other researchers examined same or other clinical bacteria (*Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enteritica,* and *Staphylococcus aureus*), as well as on the other virulence factors like bacteria cell elongation (Table 4) [31-33].

**Table 4:** Anti-biofilm activity of aquatic extracts of *Theobroma cacao* against gram-negative bacteria

| Gram negative bacteria | *Theobroma cacao* |
|---|---|
| *Salmonella typhi* | High |
| *Salmonella typhimurum* | High |
| *Acinetobacter* | High |
| *Escherichia coli* | High |
| *Serratia spp.* | High |
| *Pseudomonas aeroginosa* | Moderate |
| *Pseudomonas flourscences* | Moderate |
| *Proteus vulgaris* | Moderate |
| *Proteus merabilis* | Moderate |
| *Klebsiellap neumoniae* | Moderate |
| *Enterobacter aerugenes* | Moderate |

## Conclusion

*Theobroma cacao* (30%) was appeared in this research as high efficient against wide spectrum of clinic isolates gram-negative and gram-positive bacteria (*K.pneumonia, P.merabilis, P.vulgaris, S.typhimurum, S.typhi*, *Staph.aureus, Staph.epidermidis, Staph.saprophytics, and Strep.Pyogenes*) which indicates that it is more effective than commercially available antibiotic in addition to the high inhibition against adherents and biofilm formation.

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## Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

## Conflict of Interest

There are no conflicts of interest in this study.

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