THE ANTIBACTERIAL EFFECT OF HERBAL ACACIA EXTRACTS ON STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS BACTERIA: AN IN VITRO STUDY

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ABSTRACT:

Objective: The aim of this an in vitro study was directed to compare the anti-bacterial effects between herbal extract of Acacia fruits and Acacia leaves on Streptococcus mutans and Lactobacillus acidophilus bacteria.

Subjects and methods: 90 samples of tested material (acacia fruits and acacia leaves extracts) and control group were equally divided into 3 groups. The 30 samples of each group were subdivided into 2 equal subgroups; one to test the material antibacterial against Streptococcus mutans and the other to test the material antibacterial against Lactobacillus acidophilus bacteria.

Results: It was found that Amoxicillin have the highest antibacterial effect with highly significant difference in compared to other groups. While acacia fruits came second followed by acacia leaves respectively. However there are high significant differences between all groups except that between acacia fruits and acacia leaves against Lactobacillus acidophilus bacteria which showed equal antibacterial activity.

Conclusion: Amoxicillin still has the highest antibacterial effect. Although the Acacia nilotica fruits and leaves extracts had higher antibacterial activity; further studies and modifications are needed before use of these extracts as antibacterial in humans.

Keywords: Acacia nilotica fruits, Acacia nilotica leaves, Lactobacillus acidophilus, Streptococcus mutans.

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INTRODUCTION

Dental caries is a complex, long-lasting, avoidable, and confined contagious condition that happens when the host, bacteria, food, and time interact, leading to the loss of minerals from the enamel and dentin. It can harm children's well-being by causing pain, poor nutrition, and early loss of teeth and affect their growth and development. It is an infectious condition caused by the existence of oral bacteria, mainly Streptococcus mutans for its start and lactobacillus for its advance (1).

Streptococcus mutans is a bacterium that is Gram-positive, nonmoving, round-shaped, and can live with or without oxygen. It sticks to the tooth surface in the dental plaque biofilm and helps the start and advance of tooth decay(2). S. mutans causes disease by making acid when there is dietary sucrose and being able to survive in acid, which support changes in the dental plaque ecology by selecting for a flora that causes tooth decay, increasing the chance of enamel loss, and, finally, leading to tooth decay(3).

Mechanical methods such as toothbrushing and flossing, along with chemical agents like mouthwashes, can be employed to ward off decay by eliminating microbes. However, these chemical agents can disrupt the mouth's natural balance and potentially lead to microbial resistance(4). Given the increasing resistance of disease-causing microbes to chemical antimicrobials and the side effects associated with these compounds, researchers have turned to medicinal plants in search of new antibacterial sources(5). These plants are abundant in active biological compounds that exhibit potent antibacterial properties. Phytoalexins, small antibiotics with a molecular weight of less than 500, are primarily responsible for the antimicrobial activity in medicinal plants. They are categorized into several groups, including polyphenols, flavonoids, terpenoids, and glycosteroids(5).

The Acacia genus, second only in size within the Leguminosae family, boasts approximately 1350 species. These species are predominantly found in the world's tropical and warm temperate regions, with Australia, The Americas, Africa, and Asia being home to 957, 185, 144, and 89 species respectively. Acacia nilotica, one of these species, has been traditionally used in the treatment of various ailments such as tuberculosis, leprosy, smallpox, dysentery, cough, eye infections, toothache, and skin cancer. It has also been used as an astringent, antispasmodic, and aphrodisiac by rural communities(6).

In recent times, there has been a surge in efforts to discover new plant-based therapeutic strategies against oral microbes. This is largely due to the presence of secondary metabolites in plants, which have significant pharmacological effects against various microorganisms and exhibit synergistic patterns(7). Acacia nilotica is known to contain alkaloids, saponins, cardiac glycosides, tannins, flavonoids, and anthraquinones. These phytochemical constituents are primarily responsible for the antimicrobial efficacy of Acacia nilotica(8).

Acacia nilotica has been found to be effective against S. mutans, demonstrating its antimicrobial potency. Furthermore, in vitro studies have shown that the methanolic and acetone extracts of Acacia nilotica can inhibit the growth of L. acidophilus(9).

Therefore, the present study was conducted to test the antimicrobial activity of herbal product Acacia nilotica to develop agents with antimicrobial therapeutic potential to ascertain the rationale for their use in dentistry.

Subjects and methods:

Study design: Prospective In-vitro study.

Study setting: It was done at the bacteriology lab (for about six months or more than this period) at botany and microbiology department, faculty of science, Al-Azhar University, Boys, Cairo.

Ethical consideration: The research protocol is ethically accepted with code: 525/3046 at 19/09/2020 by committee of faculty of Dental Medicine, Boys, Cairo, Al -Azhar University.

Source of reference Bacterial Strains:

Streptococcus mutants: ATCC 25175.

Lactobacillus acidophilus: DSM 20079.

Prepared at Microbial Resource Center, faculty of agricultural Ain Shams University.

Media used:

1) Nutrient Agar (NA) Medium: (10)

Used in cultivation and enrichment culture media for Streptococcus mutans and Lactobacillus acidophilus.

2) Muller-Hinton Agar (MHA): (11)

This media is used in antimicrobial susceptibility testing. This formula conforms to Clinical and Laboratory Standard Institute (CLSI) (12).

Grouping:

This study consisted of 104 sample and divided into four main groups according to type extract:

Group A: Acacia fruits extract was dissolved in ethyl acetate.

Group B: Acacia leaves extract was dissolved in ethyl acetate.

Group C: Amoxicillin (Bioanalyse company) was considered as a (control group).
Each group was divided into two subgroups according to types of bacteria investigated (S.mutans and Lactobacillus acidophilus).

Intervention:
Collection of plant material:
Acacia nilotica Fruits and Acacia nilotica Leaves were collect in spring 2020, identified, prepared and investigated in Botany and Microbiology Department, Faculty of Science, Al-Azhar University".
Preparation of Acacia nilotica fruits and Acacia nilotica leaves extracts:
The dried powder of Acacia nilotica Fruits and Acacia nilotica leaves were extracted in Ethyl acetate solvent for 72hr on rotary shaker by cold percolation method (13). The following steps were taken to extract the plant material; 10 grams of dried powder were mixed with 100 ml of solvent in conical flask, covered with cotton wool, and left for 72hr. Then, the extract was passed through 8 layers of muslin cloth. The filtrate was spun at 5000 rpm for 10 min. The supernatant was dried using rotary evaporator. The dried extract was stored at 4°C in air-tight bottles.

Observation:
Screening of Antibacterial activity of Plants extracts on Streptococcus mutans and Lactobacillus acidophilus:
1) Plate Seeding with Streptococcus mutans and Lactobacillus acidophilus:
A full loop of Streptococcus mutans ATCC 25175 and Lactobacillus acidophilus DSM 20079 were introduced into 25 ml of Muller Hinton broth in a 150 ml conical flask. This was then left at room temperature on a rotary shaker for 24 hours to activate the test bacteria, achieving a final cell concentration of 1×108 CFU/ml. The Mueller Hinton Agar was then combined with 200µl of the bacterial mixture and thoroughly mixed to ensure even distribution across the plate(14).
For the antibacterial screening using the Agar Well Diffusion Method, plant extracts were dissolved in 1% dimethylsulphoxide (DMSO) for the antibacterial study, with the extract concentration adjusted to 100 mg/ml(14). The antimicrobial activity of the plant extracts against Streptococcus mutans and Lactobacillus acidophilus was measured using the Agar well diffusion method. Wells of 6 mm were created using a sterile corks borer, and 100 µl of crude extracts were added to each well individually and left for 2 hours at 4°C. Amoxicillin was used as a control for the bacterial strain. The plates were then incubated for 24 hours at 37°C, after which the inhibition zones were measured and recorded.

Statistical analysis
Data was analyzed using SPSS (statistical package for social sciences) version 22. The suitable statistical test was used according to data type with the following suggested tests: One-way ANOVA test was used to compare among the four groups. The post-Tukey's test was used.

RESULTS:
Comparison of the antibacterial activity of Acacia fruits, Acacia leaves and Amoxicillin (control) on Streptococcus mutans strain:
Concerning the antibacterial activity of different materials against Streptococcus mutans strain, it was found that Amoxicillin (control group) have the highest antibacterial effect with highly significant difference in compared to other groups. While acacia fruits came second followed by acacia leaves respectively. However there are high significant differences between all groups (Tab. 1 Fig. 1).

Table (1): The antibacterial activity of Acacia fruits, Acacia leaves and Amoxicillin (control) on Streptococcus mutans strain.

Materials	N	Mean (mm)	Std. Devi (±)	Min.	Max.	Mean Difference(±)	Sig.
1. Acacia fruits	**15**	25.0769	.86232	24.00	26.00	**1:2** = 1.61538****	**0.000****
2.Acacia leaves	**15**	23.4615	.96742	22.00	25.00	**1:3** = 7.76923****	**0.000****
3. AX (control)	**15**	30.2308	.72501	29.00	31.00	**1:4**= 5.15385**	**0.000****

Anova test.
*Significant at P ≥ 0.05
**Highly significant at P ≥ 0.01

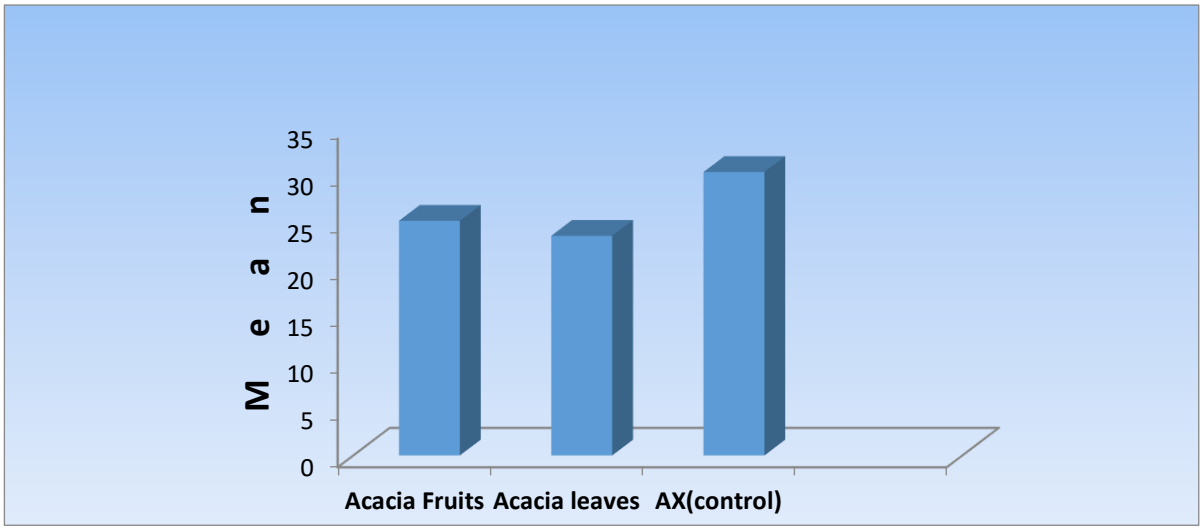

Figure (1): Diagram showing Comparison of antibacterial activity of Acacia fruits, Acacia leaves and Amoxicillin on Streptococcus mutans strain.

Comparison of the antibacterial activity of Acacia fruits, Acacia leavesand Amoxicillin (control) on Lactobacillus acidophilus strain:

In relation to antibacterial activity against Lactobacillus acidophilus strain was found that Amoxicillin have the highest antibacterial effect with highly significant difference in compared to all other groups. While results showed equal antibacterial activity of acacia fruits and leaves hence there is no significant difference between them. (Tab. 2 Fig. 2).

Table (2): The antibacterial activity of Acacia fruits, Acacia leaves and Amoxicillin (control) on Lactobacillus acidophilus strain.

Materials	N	Mean (mm)	Std. Devi (±)	Min.	Max.	Mean Difference(±)	Sig.
1. Acacia fruits	**15**	20.5385	.96742	19.00	22.00	**1:2** = .00000	**1.000**
2.Acacia leaves	**15**	20.5385	.96742	19.00	22.00	**1:3** = 5.23077**	**0.000****
3. AX(control)	**15**	29.3077	.85485	28.00	31.00	**1:4** = 8.76923**	**0.000****

Anova test.

* Significant at P ≥ 0.05

** Highly significant at P ≥ 0.01

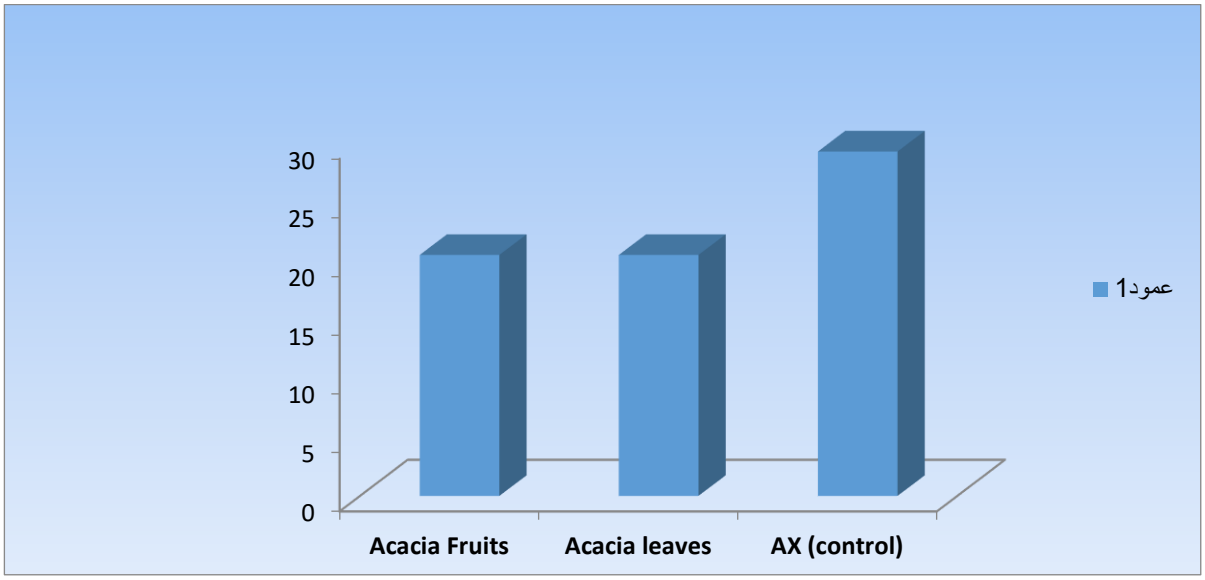

Figure (2): Diagram showing Comparison of antibacterial activity of Acacia fruits, Acacia leaves and Amoxicillin on Lactobacillus acidophilus strain.

DISCUSSION:

There is a need for the development of novel natural antimicrobial biocompatible drugs since the excessive and illogical use of antibiotics has led to the development of multidrug-resistant pathogens (15). Herbal products have been utilized in traditional medicine for ages and predate the invention of antibiotics and other contemporary medications(16). Further research into plant-based antimicrobials is necessary since they represent a sizable unexplored supply of medications and have a great deal of therapeutic potential. They are biocompatible since effectively combat of the negative effects of synthetic antimicrobials while also being effective in treating infectious infections (17).

The results of this present study revealed that the fruits of Acacia nilotica and leaves have a significant high antimicrobial effect. This could be attributed to the existence of polyphenolic, flavonoids, and reactive oxygen species (ROS) in fruits and leaves of Acacia nilotica (18,19). These results are in agreement with the results of the study by Sadiq et al. (18) who concluded that all parts of the Acacia nilotica plant were found to be effective even against the antibiotic-resistant and sensitive pathogens.

Also, the results of this study revealed that the fruits of Acacia nilotica has a significantly higher antimicrobial effect than its leaves specially against S mutans. This can be explained by the presence of phenolic and flavonoid compounds at diffident percentages according to plant species, age, growing conditions, soil conditions, and post-harvest treatment (20). Moreover, Pereira et al. (21), claimed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their robust bioactive qualities. This explains why a mix of natural phytochemicals of Acacia nilotica fruits is able to produce a higher antibacterial effect. These results are following the results of Singh et al. (19) who concluded that the presence of phenolics was evident in all leaves and fruits extracts, and the fruits extract had the highest total phenolic concentration in comparison with their leaves. Moreover, the authors found a significant association between antioxidant activity and total phenolic levels in the current study. Moreover, Sachir et al. (22) stated that polyphenols and flavonoids are present in all plant extracts, and their concentration is correlated with their antibacterial action.

Furthermore, the results of the current investigation revealed that the fruits of Acacia nilotica has a significantly higher antimicrobial effect (higher inhibition zone) followed by Acacia nilotica leaves specially for S. mutans strain. These results are in agreement with the results of Sadiq et al. (18) study who concluded that all parts of the Acacia nilotica plant were found to be effective even against the antibiotic-resistant and sensitive pathogens.

Conclusion: According to the finding of this current investigation, it could be concluded that Amoxicillin still have the highest antibacterial effect. Moreover, the Acacia nilotica fruits of ethyl acetate extract had higher antibacterial activity specially against S.mutans compared to Acacia nilotica leaves an. Further modification and studies (in vivo and in vitro) are needed before use of these extracts as antibacterial in humans.

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