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THE EFFECT OF OLIVE LEAVES AND 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 & leaves and olive leaves on Streptococcus mutans and Lactobacillus acidophilus bacteria. **Subjects and methods:** 104 samples of tested material (acacia fruits and acacia leaves and olive leaves extracts) and control group were equally divided into 4 groups. The 26 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 all other groups. While acacia fruits came second followed by acacia leaves and Olives 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 compared to Olive leaves extracts; but further studies and modifications are needed before use of these extracts as antibacterial in humans.

KEY WORDS: Acacia nilotica fruits, Acacia nilotica leaves, Olive leaves, Lactobacillus acidophilus, Streptococcus mutans.

INTRODUCTION

Dental caries is a multifactorial, chronic, preventable, and localized transmissible disease as a result of interaction among host, bacteria, diet, and time, causing cavitation of inorganic components of the enamel and dentin. It can affect children's life quality by causing pain, malnutrition, and premature loss of teeth and alters the growth and development. It is an infectious disease caused by the presence of oral bacteria mainly Streptococcus mutans for its initiation and lactobacillus for its progression [(1)].

Streptococcus mutans is a Gram-positive, nonmotile, coccus-shaped, and anaerobic facultative bacterium that is found in the human oral cavity. It adheres to the tooth surface in the dental plaque biofilm and favors the initiation and progression of dental caries[(2)]. Pathogenicity of S. mutans occurs as a result of its acidogenicity in the presence of dietary sucrose and its accompanying acid tolerance, together support changes in the dental plaque ecology by choosing for a cariogenic flora, raising the enamel demineralization probability, and, eventually, formation of dental caries[(3)].

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Decay prevention includes mechanical methods (toothbrush–dental floss) and the use of chemical agents with antimicrobial features, such as mouthwashes. The use of chemical agents may lead to microbial resistance and can also cause certain complications by disturbing the biological equilibrium of the mouth[4]. In recent years, due to the increasing resistance of pathogenic microorganisms to the chemical antimicrobials and the side effects of these compounds, researches on medicinal plants have been considered in order to discover new antibacterial sources[5].

Medicinal plants are a rich source of active biological compounds with advanced antibacterial properties. The most known cause of antimicrobial activity in medicinal plants is the presence of phytoalexins compounds, which are small antibiotics with <500 molecular weight, and are divided to several groups including polyphenols, flavonoids, terpenoids, and glycosteroids[5]. Olive leaves has been widely used in folk medicine over centuries. Olive leaves extract contains a noticeable amount of polyphenols, which is responsible for its various medicinal properties[6].

The genus Acacia is the second largest in the family Leguminosae, with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world, with the largest concentration of species in Australia (957 species), The Americas (185 species), Africa (144 species), and Asia (89 species). Out of these, Acacia nilotica is one of the species that has been effectively utilized in folk medicine for the treatment of tuberculosis, leprosy, smallpox, dysentery, cough, ophthalmia, toothache, skin cancer as astringent, antispasmodic, and aphrodisiac by rural population[7]. Recently, efforts have been increased to identify new therapeutic strategies against oral microbes, using plant compounds. Regarding the presence of secondary metabolites, plants have significant pharmacological effects toward different microorganisms and their synergistic patterns[8].

Therefore, the present study was conducted to test the antimicrobial activity of herbal products of Olive and 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: [9]

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

2) Muller-Hinton Agar (MHA): [10]

This media is used in antimicrobial susceptibility testing. This formula conforms to Clinical and Laboratory Standard Institute (CLSI) [11].

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: Olive leaves extract was dissolved in ethyl acetate.

Group D: 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 Leaves) and Olive leaves were collected in spring 2020, identified, prepared and investigated in Botany and Microbiology Department, Faculty of Science, Al-Azhar University"**.**

Preparation of Acacia nilotica (fruits and leaves) and Olive leaves extracts:

The dried powder of Acacia nilotica (Fruits and leaves) and Olive leaves were extracted in Ethyl acetate solvent for 72hr on rotary shaker by cold percolation method[12]. The extraction process was run as follow; 10 grams of dried powder were added to 100 ml of solvent in conical flask, plugged with cotton wool, and kept for 72hr. Then, the extract was filtered with 8 layers of muslin cloth. The filtrate was centrifuged at 5000 rpm for 10 min. The supernatant was evaporated 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 loop full of Streptococcus mutans ATCC 25175 and Lactobacillus acidophilus DSM 20079 were inoculated in 25ml of Muller Hinton broth in 150ml conical flask and incubated at room temperature on a rotary shaker for 24hr to activate the test bacteria.

The final cellular concentration was 1×10^8 CFU/ml. The Mueller Hinton Agar was inoculated with 200µl of bacterial suspension and tilted well to ensure proper distribution in the plate[13].

2) Antibacterial Screening by Agar Well Diffusion Method:

Plant extracts were dissolved in 1% dimethylsulphoxide (DMSO) for antibacterial study. Extract concentration was adjusted to 100 mg/ml[14]. The Agar well diffusion method was performed for the determination of antimicrobial activity of plant extracts against (Streptococcus mutans and Lactobacillus acidophilus).

Wells (6 mm) were cut using a sterile corks borer; 100 μl of crude extracts were transferred to each well individually and left for 2h at 4°C. Amoxicillin was used as a control for bacterial strain and then, the plates were incubated for 24 h at 37°C. After incubation, the inhibition zones were measured and recorded.

Statistical analysis

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

RESULTS

Comparison of the antibacterial activity of Acacia fruits, Acacia leaves, Olive 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 all other groups. While acacia fruits came second followed by acacia leaves and Olives 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, Olive leaves and Amoxicillin (control) on Streptococcus mutans strain.

Materials	N	Mean (mm)	Std. Devi (±)	Min.	Max.	Mean Difference (±)	Sig.
1. Acacia fruits	**13**	25.0769	.86232	24.00	26.00	**1:2 =** 1.61538**	**0.000****
						1:3 = 7.76923**	**0.000****
2.Acacia leaves	**13**	23.4615	.96742	22.00	25.00	**2:3 =** 6.15385**	**0.000****
						1:4 = 5.15385**	**0.000****
3. Olive leaves	**13**	17.3077	.85485	16.00	19.00		
4. AX (control)	**13**	30.2308	.72501	29.00	31.00		

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

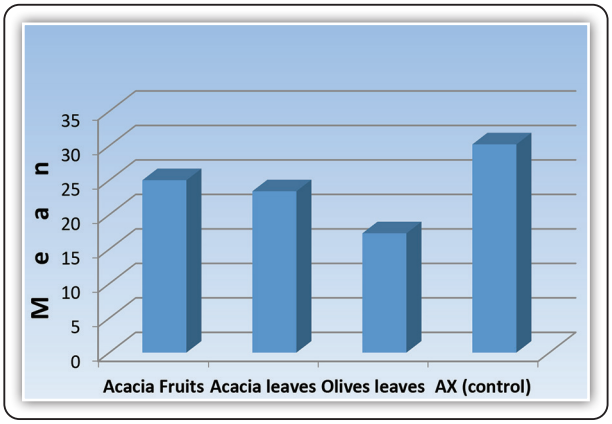

FIG (1) Diagram showing Comparison of antibacterial activity of Acacia fruits, Acacia leaves, Olive leaves and Amoxicillin on Streptococcus mutans strain.

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

In relation to antibacterial activity against Lactobacillus acidophilus strain was found that Amoxicillin has 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. However the olive leaves extract showed the lowest antibacterial activity against Lactobacillus acidophilus strain with high significant differences with all other groups (Tab. 2 Fig. 2).

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

Materials	N	Mean (mm)	Std. Devi (±)	Min.	Max.	Mean Difference (±)	Sig.
1. Acacia fruits	**13**	20.5385	.96742	19.00	22.00	**1:2 =** .00000	**1.000**
2.Acacia leaves	**13**	20.5385	.96742	19.00	22.00	**1:3 =** 5.23077**	**0.000****
3. Olive leaves	**13**	15.3077	.75107	14.00	16.00	**2:3 =** 5.23077**	**0.000****
4. AX (control)	**13**	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*

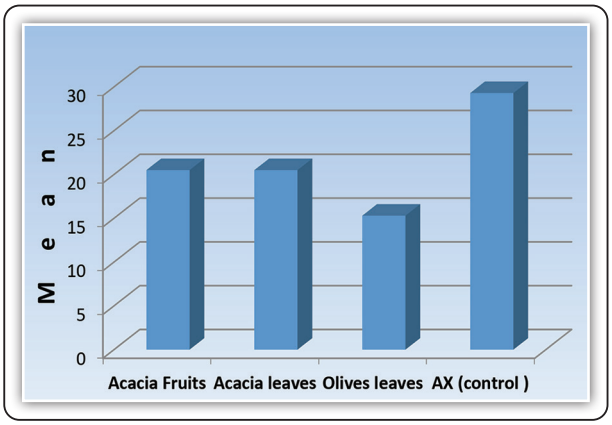

FIG (2) Diagram showing Comparison of antibacterial activity of Acacia fruits, Acacia leaves, Olive leaves and Amoxicillin on Lactobacillus acidophilus strain.

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 findings of the present investigation demonstrated that S. mutans and Lactobacillus growth is inhibited by ethyl acetate extract of the Olive leaves. In agreement with the results of this study, the results of the study by Vural and Akay [18] and Rafiei et al. [19] on Olive leaves extracts were found that have antimicrobial activities against different bacterial and fungal strains.

This may be explained by the fact that olive leaves extracts, which contain phenolic chemicals like oleuropein and hydroxytyrosol, have strong antibacterial activity [20]. Moreover, the presence of Anti-quorum sensing (QS) chemicals and other plants is likely the cause of the bacteria's cell wall being destroyed by Olive extracts. Thymus deanesis, Thymus Vulgaris, thymol, and carvacrol are substances presents in olive leaves extract have antibacterial activity against S. mutans and Lactobacillus according to Gray et al. [21] and Khozeimeh et al.[22].

Moreover, 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 [23, 24]. These results are in agreement with the results of the study by Sadiq et al. [23] 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 [25]. Moreover, Pereira et al. [26], 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 can produce a higher antibacterial effect. These results are following the results of Singh et al. [24] 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. [27] 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 and then by Olive leaves specially for S. mutans strain. These results are in agreement with the results of Sadiq et al.[23] study who concluded that all parts of the Acacia nilotica plant were found to be effective even against the antibiotic-resistant and sensitive pathogens. However, this study came in harmony with the study of Vural and Akay [18] who demonstrated that the Olive leaves' essential oil showed lower, tolerable antioxidant and antimicrobial activity.

CONCLUSION

According to the finding of this 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 and Olive leaves extracts. Further modification and studies (in vivo and in vitro) are needed before use of these extracts as antibacterial in humans.

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