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Abstract- *Background:* When root canal infections are concerned, the causative factor is primarily the microbial inhabitation, which leads to the spread of infection. The literature evidence supports the aspect that frequent usage of antibiotics induces resistance and threatens the effectiveness of the treatment. So, the current issue that is faced globally is the development of resistant microbial species. To combat this interest, towards the usage of newer herbal horizons is increased.

Materials and Methods: The antibacterial effect of Ethanolic Leaf Extract of Morinda Citrifolia was investigated against Enterococcus Faecalis (E. Faecalis). Agar well diffusion and broth dilution methods were used to determine the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Results: The MIC of Ethanolic Extract of Morinda Citrifolia extract was found to be 250 µg/ml, and the MBC was 400 µg/ml

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Assessment of Antibacterial Activity and Minimal Inhibitory Concentration of Leaf Extract of Morinda Citrifolia Against Enterococcus Feacalis An Invitro Study

Venkata Teja Kavalipurapu [α], Apoorva Vasundhara Kaligotla [σ] & Gummuluri Sriram [ρ]

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Conclusion: Ethanolic leaf extract showed better antimicrobial activity on E. Faecalis.

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I. Introduction

Endodontics is a field where complex root canal anatomy, dealt in a simplified way. Most of the treatment and therapeutic success attributed to the complete removal of tissues and contents from root canal space, including the dentinal and microbial debris (1),(2). When root canal infections are concerned, the causative factor primarily is microbial inhabitation, leading to the spread of infection into periapical space, causing apical periodontitis(3). It's a known fact that root canal infections cannot be attributed to a single species or a single microbe. The pathogenic resistant microbial colony is attributed to the formation of a complex biofilm(4),(5). So, the major hurdle in therapeutic endodontics is the complete removal of bacterial biofilm(5).

So, from the previous discussion, it's understood that microbial inhabitancies are primary causation factors for root canal infections. Thus, therapeutic options and advancements tend to concentrate more on this aspect. Although the advancing front is towards the discovery of antimicrobial agents in eradicating the bacterial biofilm, it's more neglected that the threat of antimicrobial resistance is also increasing. So, the current issue that is faced globally is the development of resistant microbial species(6). The literature evidence supports the aspect that, frequent usage of antibiotics induces resistance and threatens the effectiveness of the treatment (7),(8).

The evolving trend in new therapeutics is concentrating on a wide range of herbal products and natural plant extracts. The evidence also supports that these products exhibit a broad range of antimicrobial properties and therapeutic benefit(9),(10). So, many researchers and clinicians have shifted their interest in exploring the natural plant extracts and assessing their therapeutic effects. Although there is a wide range of natural products and plant extracts widely used for various medicinal purposes, the well-documented usage and benefits in endodontics are seen majorly with Morinda Citrifolia(11).

A study by Murray et al.,(12) stated that, the antimicrobial efficacy of Morinda Citrifolia juice was similar to 6% sodium hypochlorite, which is considered as a gold standard root canal disinfectant in endodontics. The currently available evidence is strongly in favor of sodium hypochlorite as the main endodontic irrigant(13).

The concentration of hypochlorite used and the contact time of hypochlorite with root canal walls deals with its effectiveness in reduction of microbes (14), especially E. Faecalis, which is known to be a resistant species responsible for the reinfections(15). But, the sad truth is that the choice of irrigant concentration and usage cannot be standardized in the clinical scenario as it is possible in an experimental scenario. So, in a clinical scenario, various studies compiled and showed evidence of developing a resistant E. Faecalis in failed primary root canal treatments(16),(17). Hypochlorite at lower concentrations might lead to the development of resistant strains (14).

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So, this was the primary concern for the authors of the present study. We state that "Herbal products although might not replace primary root canal irrigant, but might be an adjunctive therapeutic option and a lot of research and therapeutic potential, still lack and to be translated in a clinical scenario". So, our research majorly concentrates on assessing various plant extracts. Our previous research has focused on assessing the Ethanolic Fruit Extract of Morinda and proved to be beneficial against E. Faecalis(9). So, the present study formulated was to assess a step ahead to evaluate the beneficial antimicrobial effect of Ethanolic Leaf Extract of Morinda Citrifolia. The null hypothesis stated was, there was no significant antibacterial effect of ethanolic leaf extract of Morinda Citrifolia against E. Faecalis on using agar well diffusion and broth dilution methods.

II. Materials and Methods

The study was approved by the Institutional Ethical Committee. Before the start of the research, the sun-dried powder of Morinda Citrifolia was collected. The extract preparation was similar to our previous study(9), where we used the dried fruit and seeds powder against E.Faecalis. In this present study, we concentrated mainly on the effect of dried leaf powder. The dried leaf powder was initially subjected to 1000ml of ethanol using Soxhlet extractor for 72, not exceeding the boiling point of the solvent. After which, the extract was filtered and vacuum dried at 45 degree Celsius. The obtained extract was refrigerated until use.

The strains used for testing were gram-positive E. Faecalis bacterial strains ATCC 29212. The bacterial strains were cultured in Luria broth agar (Himedia, Mumbai) and incubated at 37-degree celsius for 24 hours and maintained on nutrient agar slants at 4-degree celsius. The sterile spreader was used for the inoculation of these organisms across the media. 10mg/ml of sample was provided for analysis.

Determination of Minimum Bactericidal Concentration:

The microorganisms were inoculated into molten Luria broth agar and poured into Petri dishes and solidified. Wells of uniform diameter were then prepared on the solidified agar. The discs were then impregnated with experimental test solution at different concentrations of 40, 60, 80, and 100μl, respectively. 10mg/ml of tetracycline was used as a positive control, and the solvent without plant extract was used as a negative control. Plates were incubated for 24 hours at 37-degree celsius, and the development of the inhibitory zone around the wells was measured in diameter and recorded.

MBC value was determined by subculturing the test dilution on a freshly prepared nutrient agar media. The plates were incubated further for 18-42 hours at 37-degree celsius. The highest dilution yielded no visible turbidity on nutrient agar and taken as MBC.

Determination of Minimum Inhibitory Concentration:

The MIC of the test solutions was determined using broth dilution methods using CLSI 2012 standard protocol (18).

The cultures were then incubated and subsequently, serially diluted to reach a density of 2×104 cells per ml. Cell counting was done using a hemocytometer. Luria broth (Himedia, Mumbai) was prepared and sterilized at 121-degree celsius, 15lbs for 15 minutes.

Two milliliters of Luria broth was dispensed into the tubes, and 100μL of cell culture was inoculated in it. Different concentrations of ethanolic leaf extracts of 7.5, 15.625, 31.25, 62.5, 125, 250, 500, and 1000μg respectively were added into the tubes. Positive and negative controls were similar to agar well diffusion. Growth control was run parallel with every experiment. All the experimental tubes were incubated for 48 hours in anaerobic jars. After completion of the incubation period, the optical density was measured at 600nm. Each experiment was carried out in a triplicate set. The lowest possible concentration before the color change was considered as MIC. The percentage of bacterial inhibition was computed by an equation, as mentioned in our previous study(9).

III. Statistical Analysis

Data were analyzed using SPSS version 11. Multiple comparisons were made using one way ANOVA followed by LSD test for post hoc analysis. Statistical significance was considered for $p<0.05$.

IV. Results

Table 1 depicts the MBC, and Table 2 illustrates the MIC values. Each value represented as a mean ± standard deviation. When results were compared, the statistically significant difference was noted as compared to the negative control.

V. Discussion

From the results of the present study, it can be concluded that the ethanolic extract of morinda citrifolia leaf also seemed to have an efficient antibacterial activity on assessing using agar well diffusion and broth dilution methods. None of the previous literature on this specific aspect can be compared, as much of the literature is concentrated on the pathogenic medical microorganisms. When the results of the present study were compared with our previous study results, they showed inferior values on both MIC and MBC evaluation. So, it can be assessed that fruit extract seemed to have better antimicrobial properties as compared to leaf extract on ethanolic extraction.

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Previous literature has reported the potent antimicrobial activity in the organic solvent as compared to water as an extracting compound, which indicated that active compounds resulting in antibacterial activity are more soluble in organic solvents (19). When the antimicrobial property of the herbal or natural plant products is evaluated critically, the enhanced effect cannot be attributed to a single compound or agent. It's a combined action of various bioactive compounds such as alkaloids, flavonoids, and other significant compounds promoting its activity (20).

Previous literature on morinda citrifolia has shown enormous evidence based data on its antimicrobial, antifungal, antioxidant, and anticancer properties (21), (22), (23). As discussed previously, the combined action of bioactive compounds in the plant extract of morinda citrifolia, which led to its enhanced antibacterial activity. The null hypothesis of the present study rejected, and results proved that leaf extract of morinda citrifolia possessed antimicrobial property on assessment.

When a limitation of the present study is considered, it's a first invitro study simulated to assess a single pathogen. But, when root canal infections are considered, it's usually polymicrobial inhabitations (24). So, the effectiveness of the agent tested by this in-vitro study is not possible. So, better future studies should concentrate on combined agents on multiple endodontic pathogens to prove their effectiveness. In our perspective, there is still a long way for the natural plant extracts to be considered effective in clinical endodontics.

VI. Conclusion

Ethanolic leaf extract of morinda citrifolia seemed to possess antimicrobial properties against E. Faecalis and can be considered as an antimicrobial agent to treat root canal infections.

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