

Evaluation of Antimicrobial Activity of *Coleus forskohlii* on Periodontal Pathogenic Bacteria, An experimental in-vitro study

Amel Ibrahim Faragalla (✉ amelfaraj@hotmail.com)

King Khalid University

Ghada Khalid Bahamdan

King Khalid University

Aseelah Abdullah

King Khalid University

Raida Almoadi

King Khalid University

Mohammed Fadul

King Khalid University

Tagreed Majrashi

King Khalid University

Hasinah Algarni

King Khalid University

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Abstract

Background:

Coleus Forskohlii (C.F.) is a typical indigenous medicinal plant from the Lamiaceae family grown widely in India. *Coleus* gained importance after discovering its therapeutic properties (Bone, 2007) and was reported to have some antibacterial activity. The current study tested the antibacterial effect of (C F) on certain periodontal pathogens.

Methods

An experimental Invitro-study was conducted at King Khalid University, to test the antibacterial activity of *Coleus Forskohlii*. Thirty plaque samples were collected from gingivitis patients, kept, and transported using nutrient broth to the microbiology lab. The (CF) extract was prepared from the plant's leaves and five solutions were produced (water, methanol, ethyl acetate, chloroform, and hexane). Following the bacterial culturing, the extract solutions were applied and the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were measured.

Results

Various activities were demonstrated, indicating the positive inhibitory reactions of ethanolic extracts against many bacteria grown directly from plaque samples. Twenty-five bacterial species have been inhibited at 1g / mL concentrations. Most organisms (82.9%) have been inhibited at 1g / mL, whereas 34.3% were inhibited at 0.25 g/ mL, and only 5.7% were inhabited at 0.15 g/ mL; the latter two concentrations represented the MIC for most of the strains.

Conclusion

The ethanolic extract of *Coleus forskohlii* demonstrated effective antimicrobial activity. The ethanolic extract and water extracts showed different activities against the selected bacteria, but the ethanolic extract was superior to the water extract.

Introduction

Medicinal plants are a rich source of antibacterial agents and curatives. *Coleus Forskohlii* (C.F) is an important medicinal plant, achievable, non-toxic, and non-irritant used by humans to manage systemic diseases like respiratory and cardiac disorders and skin infections (Bone, 2007). *Coleus forskohlii* (Lamiaceae), a traditional plant of South India, is a source of bio-reductants and stabilizers (Malleswari et al., 2013). *Coleus forskohlii* stimulates cyclic adenosine monophosphate, regulates the number of enzymes, and develops some antibacterial effects (Batista et al., 1995). It has biological activities

(Caprioli et al., 1984) (Bauer et al., 1993). Some studies reported using C. F. to treat systemic cardiovascular, respiratory disorders, and congestive heart failure (Shivaprasad et al., 2014). It has antimicrobial, antioxidant, and many pharmacological activities (Shivaprasad et al., 2014). The essential oil derived from *Coleus forskohlii* showed antimicrobial activity against streptococcus species (Majeed and Prakash, 2003). Due to the presence of a wide range of periodontal pathogens and the development of bacterial resistance, the current study tested the antibacterial activity of *Coleus Forskohlii* against aerobic periodontal pathogens. It aimed to determine the *Coleus forskohlii* effect on the aerobic bacterial pathogen that initiates gingivitis by collecting plaque samples. However, to our knowledge, no previous study was conducted to test (CF) antibacterial activity against aerobic periodontal pathogens.

Methods

An experimental Invitro-study was conducted at the Periodontic Clinic, College of Dentistry, King Khalid University, Abha, Saudi Arabia. This study was approved by the University's Institutional Review Board and provided the registration number IRB/REG/2022-2023/3. The study was according to the ethical principles of the World Medical Association Declaration of Helsinki (2013). Volunteers signed the consent form before participation.

Thirty plaque samples were collected from male and female gingivitis patients seeking periodontal treatment. A sterile paper point size (40) was inserted in the gingival sulcus for 15 seconds. Then, samples were kept and transported using nutrient broth media suitable for aerobes to the microbiology lab. *Coleus forskohlii* leaves were collected from the Aseer region, Abha Kingdom of Saudi Arabia, in October 2022. The leaves were carefully rinsed with distilled water, dried overnight in a hot air oven at 40°C, and ground to a particle size of 40 mesh by using mortar and pestle. The plant powder (32.6 g) was divided into five portions, and each portion was extracted with a solvent, including water, methanol, ethyl acetate, chloroform, and hexane by decoction for two hours, followed by maceration for 48 hours to get five extracts.

Assay for the antimicrobial activity of *Coleus forskohlii* extract was done by the disk impregnation method with minor modification from the following standard methods (Breakpoints, 2011) : One to three loopful of 24 hold cultures from each test strain were used to prepare 0.5 McFarland standard suspensions. A loopful of 0.5 McFarland suspensions was used to streak Mueller Hinton's agar plates (Difco). Discs were placed on the inoculated agar, and inoculated plates were incubated at 37°C and examined after 24, 48, and 72 hours to detect the inhibition zones. Inhibition zones were measured in mm by a ruler for each bacterial strain under and around discs. The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) have been considered valuable endpoints of antibacterial activity to guide effective therapy of bacterial infections. The disc impregnated discs at various concentrations have been tested to determine the MIC.

Results

The antimicrobial activity of ethanolic and water extract of *Coleus forskohlii* against clinical and reference bacterial strains is shown in Table 1 and Figures 1, 2, and 3. Isolation and the preliminary naming standard were performed as per routine laboratory methods and following standard protocols (Guo-Xin et al., 2014). The identity of isolates was confirmed using the Vitek 2 automated system according to manufacturer procedures (bio Mérieux VITEK, Inc.). Vitek 2 was also used to test their antimicrobial sensitivities.

The ethanolic and water extracts showed different activities against the selected bacteria, but the ethanolic extract was superior to the water extract. A wide spectrum inhibitory activity was shown against beta-hemolytic *Streptococcus pyogenes*, an isolate from plaque samples. The activity against *Staphylococcus aureus* and *E. coli* was less than that against *Streptococcus pyogenes*. The direct in-vitro assay was applied to 10 clinical samples grown on Tryptocase Soy broth. The grown cultures were tested directly against the ethanolic extract of *Coleus forskohlii*. Various activities were demonstrated, indicating the positive inhibitory reactions of *Coleus forskohlii* ethanolic extracts against many bacteria grown directly from plaque samples. Direct application of an ethanolic extract of *Coleus forskohlii* indicated a good inhibitory antagonism to many unidentified bacteria grown from plaque samples. Twenty-five bacteria have been inhibited at a concentration of 1g / mL. Most (82.9%) of the organisms have been inhibited at 1g / mL, whereas 34.3% were inhibited at 0.25 g/ mL, and only 5.7% were inhibited at 0.15 g/ mL; the latter two concentrations represented the MIC for most of the strains. Table 1. In vitro assay of *Coleus forskohlii* extract against representatives gram-positive and gram-negative (zone of inhibition in mm). Figure 1. Some bacterial strains are susceptible to *E. coli* (left) and *Staphylococcus aureus* (right) to 1:10 concentration of *Coleus forskohlii* ethanolic and water extracts. Note the narrow inhibition zone of the ethanolic extract. Figure 2. Showed Susceptibility of *Streptococcus pyogenes* to 1:10 concentration of *Coleus forskohlii* ethanolic and water extracts. There was a wide inhibition zone of the ethanolic extract compared to a relatively smaller zone in the case of the water extract. Figure 3. Showed direct susceptibilities of isolates from patient gingivitis to 1:10 concentration of *Coleus forskohlii* ethanolic and water extracts. Note the narrow inhibition zone of the ethanolic extract. Note the clearance (inhibitions zone) of some plates indicating the collective action of *Coleus forskohlii* extracts against unidentified isolates (direct method).

Discussions

In the current study, five extracts of *Coleus forskohlii* were tested for antimicrobial activity against *beta-hemolytic Streptococcus pyogenes*, a Gram-positive, aerotolerant bacteria in the genus *Streptococcus*. These bacteria are extracellular and comprise non-motile and non-sporing cocci (round cells) that tend to link in chains. They are clinically important for humans, as they are infrequent but usually pathogenic (Clemons and Queen). *E. coli* and *Staphylococcus aureus* all were isolated from plaque samples. Assessed by the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). Ethanolic and water extracts showed a significant wide-spectrum inhibitory activity and reduction of the tested organism.

In agreement with this study, Pullaiah tested the activity of *Coleus forskohlii* in an oil extract against *Staphylococcus aureus*, *E. coli* *Staphylococcus aureus*, *streptococcus species*, and other aerobic bacterial pathogens and concluded with the presence of antibacterial activity of *Coleus forskohlii* to these pathogens (Clemons and Queen). Another study by Abdalrazek demonstrated the essential oil extracted from the plant's tubers, exhibiting anti-microbial properties (Abd El-Razek et al., 2020).

In agreement with the current study, Malathi study, who concluded that the ethanol extract of *Coleus forskohlii* root showed the highest antibacterial activity compared with stem and leaf. The antimicrobial activity was observed against *Klebsiella pneumonia* and *Candida albicans* in the ethanol extract. (Malathi and Rajkumar, 2015).

Kanne et al. 's research focused on the root extract of *Coleus forskohlii* (Kanne et al., 2015). In contrast, the current study focused on extracting the effective components from the Leaf part of the plant. Both parts of the plant have antibacterial activity.

Conclusion

The ethanolic extract of *Coleus forskohlii* is an effective antibacterial solution. The ethanolic extract was more effective compared to the water extract. Direct application of the ethanolic extract of *Coleus forskohlii* showed a significant inhibitory antagonism to beta-hemolytic *Streptococcus pyogenes*, *Staphylococcus aureus*, *E. coli*, and many unidentified bacteria grown from plaque samples.

Recommendation

The present study is a limited experiment but has indicated a potential activity of *Coleus forskohlii* against gram-positive and gram-negative bacteria.

Further experimentation and adjustment to this study by testing more reference and clinical pure cultures could allow getting a better conclusion and further enable in vivo application.

Abbreviations

ATCC: American Type Culture Collection. PO Box 1549, Manassas, VA 20108 USA. R, resistant; S+, sensitive narrow zone; S++, sensitive medium zone; S+++, sensitive wide zone. ND, not determined.
Microorganism Source / Code Ethanolic extract 1:10 Conc. Water extract 1:10 Conc. Positive control (Imipenem 10 µg) Negative control (normal saline) Reference bacteria strains
Staphylococcus aureus ATCC 25923 S+ R S+++ R
Streptococcus pyogenes Clinical isolate (Pharyngitis) S+ R S+++ R
E. coli ATCC 25922 S+++ R
Oral clinical samples
Sample #1 Clinical isolate (gingivitis) S+ ND ND ND
Sample #4 Clinical isolate (gingivitis) S+ ND ND ND
Sample #5 Clinical isolate (gingivitis) S+++ ND ND ND
Sample #7 Clinical isolate (gingivitis) S+++ ND ND ND
Sample #14 Clinical isolate (gingivitis) R ND ND ND
Sample #17 Clinical isolate (gingivitis) S+++ ND ND ND
Sample #30 Clinical isolate (gingivitis) S+++ ND ND ND.

Declarations

Ethics approval and consent to participate

King Khalid University, Institutional Review Board approved the study and provided the registration number IRB/REG/2022-2023/3.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests in this study.

Funding

Not applicable

Authors' contributions

Taghreed and Hasinah prepared the extract of the plant and set the five solvents. Amel I. and Ghadah performed experimental design and data analysis for antimicrobial activities of different extract concentrations. Aseela and Raida collected the plaque samples and assisted in data analysis and laboratory work, Fadul assisted in laboratory work and preparing the photographs. All authors have read and approved the final manuscript.

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Tables

Table (1): In-vitro assay of Coleus forskohlii extracts against representatives gram-positive and gram-negative (zone of inhibition in mm).

Microorganism	Source / Code	Ethanollic extract 1:10 Conc	Water extract 1:10 Conc	Positive control (Imipenem 10µ)	Negative control (Normal saline)
Staphylococcus aureus	ATCC 25923	S+	R	S+++	R
Streptococcus Pyogenes	Clinical isolate (Pharyngitis)	S+	R	S+++	R
E. Colli	ATCC 25923			S+++	R
Oral clinical samples					
Sample #1	Clinical isolate (gingivitis)	S+	ND	ND	ND
Sample #4	Clinical isolate (gingivitis)	S+	ND	ND	ND
Sample #5	Clinical isolate (gingivitis)	S+++	ND	ND	ND
Sample #7	Clinical isolate (gingivitis)	S+++	ND	ND	ND
Sample #14	Clinical isolate (gingivitis)	R	ND	ND	ND
Sample #17	Clinical isolate (gingivitis)	S+++	ND	ND	ND
Sample #30	Clinical isolate (gingivitis)	S+++	ND	ND	ND

Figures

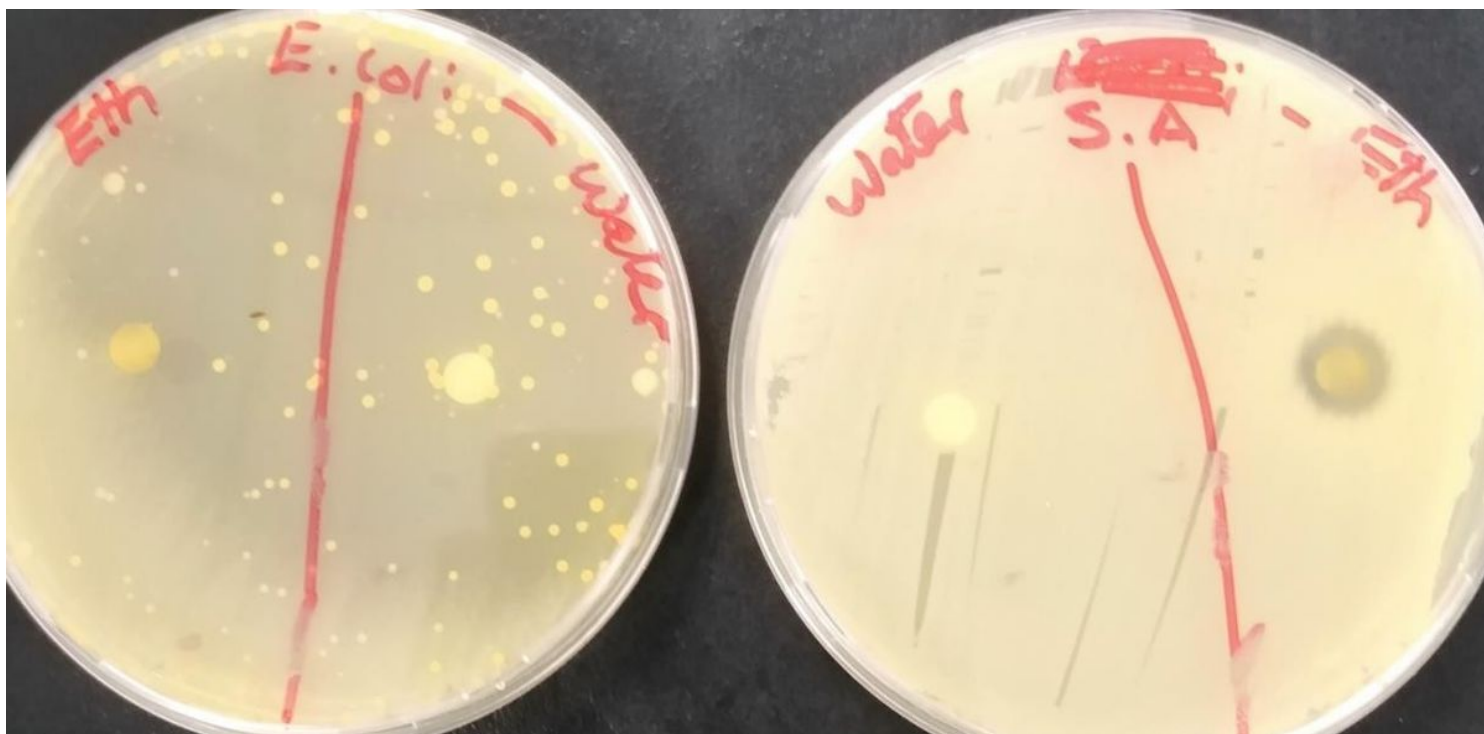


Figure 1

Some bacterial strains are susceptible to *E. coli* (left) and *Staphylococcus aureus* (right) 0 to 1:10 concentration of *Coleus forskohlii* ethanolic and water extracts. Note the narrow inhibition zone of the ethanolic extract.



Figure 2

Susceptibility of Streptococcus pyogenes to 1:10 concentration of Coleus forskohlii ethanolic and water extracts. Note the wide inhibition zone of the ethanolic extract compared to a relatively smaller zone in the case of the water extract.



Figure 3

Direct susceptibilities of plaque isolate from gingivitis patients to 1:10 concentration of Coleus forskohlii ethanolic and water extracts. Note the narrow inhibition zone of the ethanolic extract. Note the clearance (inhibitions zone) of some plates indicating the collective action of Coleus forskohlii extracts against unidentified isolates (direct method).