Antibacterial Response of *Uncaria Gambir* Leaves Extract and Ester Derivative of Catechol Against Periimplantitis Related Bacteria

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Abstract
Bacteria related periimplantitis is a serious concern for the of dental implants failure. Evidences over *S. aureus* and *E. coli* to cause periimplantitis, antibacterial potential of *Uncaria gambir* and catechol motivated present study to compare the antibacterial activity of ester derivative of catechol (EDC) and *Uncaria gambir* leaves extract (UGLE) against periimplantitis related bacteria (PRB). Current study involved synthesis of EDC and preparation of UGLE. The EDC was characterized using ATR-IR, 1H-NMR and Mass spectrometric data. Both EDC and PRB were further investigated for their antibacterial activity against PRB that is *Escherichia coli* and *Staphylococcus aureus*. Among two, the EDC exhibited high antibacterial activity when compared with UGLE. Based on the results, present study concludes that EDC possess high antimicrobial potential against PRB and recommends that EDC should be further investigated to support its clinical significance.

Keywords: Periimplantitis, Catechol, Comparison, ester, extract, and antibacterial

INTRODUCTION

During the time of implant functioning in oral cavity, dental implants (DI) are under high risk of microbial contamination, due to this more than 10 million new DI are placed worldwide annually. Peri-implantitis is a possible threat for the success of DI[1]. The human microbiome comprises 1:1 of bacteria and human cells, such that a little disturbance in this ratio may activate the bacterial periimplantitis triggering microbes (PRB)[2]. Evidence suggests two important periimplantitis related bacteria (PRB), namely *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Long-term administration of conventional antibiotics against various infections lead to potential mortality risk[3-5]. There for to combat this problem, one may use two therapeutic approaches, such as: use of synthetic or phytoproducts. Findings suggests phenolic acids and their derivatives to possess high antimicrobial potential[6-8]. Evidence suggests that plants products and extracts act as effective antimicrobial therapy[9-14], therefor these can be used for the problem of periimplantitis. Phytotherapy is an economical approach for the treatment of different diseases[15-17]. Plants elicit various biological actions, so applied in wide range of ailments and diseases like in obsessive compulsive disorder[26], antiinflammatory[27,28], antiarrhythmic[29], diabetes[30-33], hepatoprotective[34-45], antioxidant[46-49], antihyperlipidemic[60], periodontitis[50.51], antiurolithaitic[52], nephroprotective[53,54], antidepressant[55], immunity booster[56], kidney disorders[57], anticancer[58-67], antidiarrhoeal[68], digestant[69], cardiovascular disorders[59], and other pharmacological activities[70-73]. Many studies revealed increase in biological potential of plants when combined with nanotechnology[74-85]. Studies reveals several synthetic moieties to possess high antimicrobial potential[86-100]. Numerous plants product has been developed[101-118], and patented due to high biological potential[119-131]. Studies described isolation of various phytochemicals[132-171], their phyto-screening and characterization[172-177]. Therefore, present study was aimed to determine the antimicrobial potential of *Uncaria gambir* leaves extract (UGLE) and ester derivative of catechol (EDC) against PRB.

MATERIAL AND METHODS

Materials

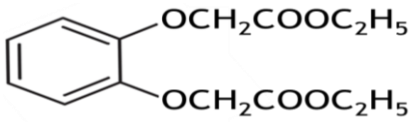
Melting points of newly synthesized compounds were determined using Thomas Hoover apparatus. IR spectra were recorded ATR-IR, Perkin Elmer, 1H-NMR on Bruker, DPX 300 and mass spectra on MASPEC (MSW/9629). Purity of synthesized compound was checked by TLC aluminium sheets – silica gel 60 F254 (0.2 mm). Plant material was collected from the local market of Sungai Petani, Malaysia. Chemicals, and solvents were procured from the SD Fine, Sigma-Aldrich, and Merck Ltd.

Preparation of Plant Extract

Preparation *Uncaria gambir* leaves extract (UGLE) was prepared as per the standard protocol[85-105]. Briefly, *Uncaria gambir* leaves free of decay or mold were collected from the province of Sungai Petani, Kedah state, Malaysia and washed with fast flowing tap water, followed by air drying, mincing into small pieces; and macerated for 15 days using hydroalcoholic solvent (50:50). The mixture was filtered using double muslin cloth and a filter paper (Whatman No. 1) and the filtrate was dried to offer dark brown colour UGLE. The obtained UGLE was stored at 4°C in refrigerator for further evaluation of its antimicrobial activity against PTB.

Procedure for the synthesis of ester derivative of catechol (EDC)

The synthesis of EDC was done as per the standard protocol with slight modifications[130-140]. Briefly, the catechol was refluxed with equimolar concentration of ethylcholoro acetate for 17 hours and purified using ether.



Response of EDC and UGLE against PRB

Preparation of bacterial culture

Bacterial strains of *Staphylococcus aureus* and *Escherichia coli* were used for the antimicrobial experiment. The prepared stock culture of microorganism was maintained at 4°C. Subcultures were prepared by transferring loopful of microorganisms' colonies from stock cultures into the nutrient broth and incubated for 24 hours at 37˚C in the incubator. The broth turbidity indicated the microbial growth[13,14].

Well Diffusion Method

The inhibitory potential of the prepared EDC and UGLE against PRB was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications[13,14]. Briefly, 20 µl of nutrient broth containing broth organism was poured into Muller Hinton agar plate, that was spread uniformly using L-shape rod. The wells were made on the agar medium with cork borer of 5 mm in diameter which was previously sterilized using autoclave at 121˚C for one hour. Each 50 µl of EDC and UGLE were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for EDC, UGLE, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the EDC and UGLE. All the plates were incubated for 24 hours at 37˚C. The diameter of zone of inhibition around wells was measured in millimetres (mm) in triplicate and average values were calculated.

Preliminary Phytochemical screening of UGLE

The UGLE was subjected to preliminary phytochemical screening for the detection of various plant constituents. The prepared extract was screened for the presence of alkaloids, carbohydrates, flavonoids, glycos - ides, proteins, tannins, and phenols as per the procedure given in standard references[175-178].

RESULTS

Synthesis of EDC

White needle shaped crystals; Yield 79%; mp 127˚C; ATR-IR: 3045, 3016, 1754 cm^{-1}; ^{1}H-NMR δ (ppm): 1.34 (3H, t, CH_3), 3.52 (2H, q, CH_2), 3.75 (2H, s, CH_2), 6.81-7.06 (4H, m, Ar-H); MS: m/z: 282 (M^+).

Response of EDC and UGLE against PRB

In present study, the prepared UGLE and evaluated for their inhibitory potential against PRB such as *S. aureus* and *E. coli* using agar

well diffusion for measurement of zone of inhibition. The prepared EDC and UGLE were evaluated for their antimicrobial potential against bacterial strains of *S. aureus* and *E. coli*. The results so obtained are given in table 1.

Table 1: Zone of inhibition of EDC and UGLE

Compound	Microorganism	Zone of inhibition			
		Reading 1	Reading 2	Reading 3	Average Value
UGLE	*E. coli*	15	15	15	15
	S. aureus	12	12	12	12
EDC	*E. coli*	22	22	22	22
	S. aureus	20	20	20	20
Streptomycin	*E. coli*	24	24	24	24
	S. aureus	25	25	25	25
Tween 80	*E. coli*	-	-	-	-
	S. aureus	-	-	-	-

Preliminary Phytochemical screening of UGLE

The UGLE was subjected to qualitative testing as per the procedure given in standard references[18,20]. The group of compounds identified in UGLE are given in table 2.

Table 2: Phytoconstituents of the CCLE

S. No.	Tests	Phytoconstituents
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Proteins	-
5	Tannins and Phenolic compounds	+
6	Sterols	+

Where, (+) positive represent presence, and (-) negative represent absence

DISCUSSION

The preliminary phytochemical screening of prepared UGLE revealed presence of alkaloids, flavonoids, glycosides, sterols, tannins, and phenolic compounds. The IR,1H-NMR, and mass spectral data of EDC was found to be in agreement with its structure. The characteristic 1H-NMR signal at 1.34 & 3.52 for CH_2 & CH_3, appearance of IR band at 1754 cm^{-1} and m/z value at 282 supported the successful synthesis of EDC. These spectral values were also further confirmed based on the previous stuties[178,179]. Evidence reports *S. aureus* and *E. coli*, to trigger microbial resistance towards conventional antibiotics raises the demand for evaluation of antimicrobials[5-8]. Research correlates the mechanics' of spread of diseases or ailments at molecular level and molecular therapeutics or approaches to treat them[181-213]. Reports suggests use of plants use the treatment of various diseases and to possess strong antimicrobial potential. Facts suggests phytochemical to elicit strong antimicrobial activity attributed to their phenolic content[214-216]. As per the literature available over different parts of *Uncaria gambir* plant and yet much more has to be explored for this plant. Hence, investigators of present study planned to evaluate the in-vitro inhibition potential of *Uncaria gambir* leaves extract against PRB (*Staphylococcus aureus* and *Escherichia coli*) using well diffusion method. The UGLE was prepared using hydroalcoholic extract 50%. The prepared UGLE was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. The UGLE showed good inhibitory effect overgrowth of *S. aureus* and *E. coli.* On the other hand, the EDC was prepared by esterification of catechol using ethyl chloroacetate and when tested against PRB (Staphylococcus aureus and Escherichia coli) exhibited high inhibitory potential study revealed that synthetic derivative (EDC) possesses high potential when compared with UGLE. However, further preclinical, and

clinical studies are required to further support the antimicrobial potential of EDC.

CONCLUSION

The results of the present study over inhibitory potential of EDC and UGLE against PRB, it is here by concluded that synthetic derivative EDC possess high antimicrobial potential against PRB especially *S. aureus* and *E. coli*. Present study recommends that highly potent EDC should be further evaluated based on the preclinical and clinical data.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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