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RESPONSE OF HYDRAZIDE DERIVATIVE OF CATECHOL AND HYDROALCOHOLIC EXTRACT OF *UNCARIA GAMBIR* LEAVES AGAINST PERIODONTITIS ASSOCIATED BACTERIA

Velmurugan Chinnasamy[1], Rajul Gupta[2], Pradeep Kumar Sharma[3]*

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Abstract

Bacteria predominance in periodontitis, antimicrobial potential of *Uncaria gambir* and catechol intended present investigation to compare the antimicrobial potential of hydrazide derivative of catechol (HDC) and hydroalcoholic extract of *Uncaria gambir* leaves (HEUGL) against periodontitis associated bacteria (PAB). Study involved synthesis of HDC and preparation of HEUGL. Synthesized compound was characterized using ATR-IR, 1H-NMR and Mass spectrometric data. Both HDC and HEUGL were further tested for their antibacterial potential against PAB such as *Escherichia coli* and *Staphylococcus aureus*. Both HDC and HEUGL exhibited high antibacterial potential against PAB, however HDC exhibited much higher antibacterial potential. Present study concludes that HDC possess high antibacterial potential against PAB and recommends that HEUGL should be further evaluated for its preclinical significance.

Keywords: Periodontitis, catechol, comparison, extract, hydrazide and antibacterial

[1]Department of Pharmacology, PGP College of Pharmaceutical Science and Research Institute, Namkkal 637001, Tamil Nadu, India
[2]Naari Pharma Private Ltd, Plot no 30, Galaxy 1st main road, JP nagar – Third Phase, Bangalore 560078, Karnataka, India
[3]*Accurate College of Pharmacy, Knowledge Park-III, Greater Noida 201306, India

***Corresponding Author** Pradeep Kumar Sharma
*Accurate College of Pharmacy, Knowledge Park-III, Greater Noida 201306, India
Email: pradeepbsr2000@yahoo.com

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INTRODUCTION

Literary evidence advocates humans' microbiota to possess almost equal ratio of bacterial and human cells[1-4]. Little change in such ratio of may manifest in various infections and diseases[5,6]. Studies suggest periodontitis as complex polymicrobial inflammatory disorder, that is associated with to dental biofilm dysbiosis that causes inflammation of periodontal tissues, followed by alveolar bone damage, and finally tooth destruction[7]. Periodontitis is associated with several other diseases, therefore a small shift in oral microbiome may be a prime factor to cause periodontitis[2]. As microbiota disturbance activates the *E. coli* and *S. aureus*[9], so at periodontal site there is high prevalence of *E. coli* and *S. aureus* [8]. Facts suggests phenols and their derivatives to possess high antimicrobial potential[6-8]. Studies suggests plants products and extracts acts as effective antimicrobial therapy[9-14], so these can be used for the problem of periodontitis. Use of phyto-therapeutics is an economical approach to treat various diseases[15-24]. Plants exhibits several biological activities, so applied in wide range of ailments and diseases such as in obsessive compulsive disorder (OCD)[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]. So, present study was designed to compare the antibacterial potential of hydrazide derivative of catechol (HDC) and hydroalcoholic extract of *Uncaria gambir* leaves (HEUGL) against periodontitis associated bacteria (PAB).

MATERIAL AND METHODS

Materials

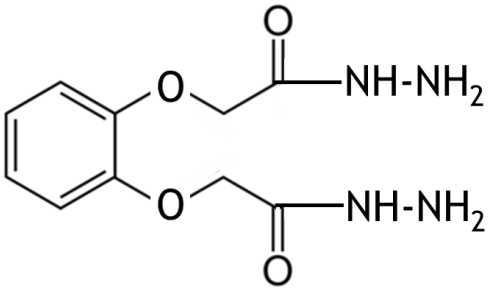
The melting point of synthesized compound was determined using Thomas Hoover apparatus. IR spectra was recorded ATR-IR, Perkin Elmer, 1H-NMR on Bruker, DPX 300 and mass spectra on MASPEC (MSW/9629). Purity of synthesized compound was checked by TLC aluminium sheets – silica gel 60 F254 (0.2 mm). Plant 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

The HEUGL was prepared as per the standard procedure in the literature[8]. Briefly, *Uncaria gambir* leaves free of decay 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 coloured *Uncaria gambir* leaves. The obtained *Uncaria gambir* leaves was stored at 4°C in refrigerator for further evaluation of its antimicrobial activity against PAB.

Procedure for the synthesis of HDC

The synthesis of HDC was done as per the standard protocol with slight modifications[86-101]. Briefly, the equimolar concentration of catechol ester was refluxed with equimolar concentration of hydrazine hydrate for 04 hours. The obtained crystals were, dried and purified using ethanol.



Response of HEUGL and HDC against PAB

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[15-20].

Well Diffusion Method

The inhibitory potential of the prepared HEUGL and HDC against PAB was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications[93-99]. 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 121ºC for one hour. Each 50 µl of HEUGL and HDC were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for HEUGL, HDC, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the HEUGL and HDC. All the plates were incubated for 24 hours at 37ºC. The diameter of zone of inhibition around wells was measured in millimetres (mm) in triplicate and average values were calculated.

Preliminary Phytochemical screening of HEUGL

The HEUGL 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, glycosides, proteins, tannins, and phenols as per the procedure given in standard references[58-59].

RESULTS

Synthesis of HDC

White crystals; Yield 76%; mp 153ºC; ATR-IR: 3259, 3012, and 1695 cm^{-1}; ^{1}H-NMR δ (ppm): 3.69 (2H, s, O-CH_2), 4.38 (2H, br, NH_2), 6.8-7.04 (4H, m, Ar-H), 9.12 (1H, brs, NH); MS: m/z: 254 (M^+).

Response of HEUGL and HDC against PAB

Prepared HEUGL and HDC were evaluated for their inhibitory potential against *S. aureus* and *E. coli* using agar well diffusion for measurement of zone of inhibition. The results so obtained are given in table 1.

Table 1: Zone of inhibition of HEUGL and HDC

Compound	Microorganism	Zone of inhibition			
		Reading 1	Reading 2	Reading 3	Average Value
HEUGL	*E. coli*	13	13	13	13
	S. aureus	16	16	16	16
HDC	*E. coli*	23	23	23	23
	S. aureus	21	21	21	21
Streptomycin	*E. coli*	24	24	24	24
	S. aureus	25	25	25	25
Tween 80	*E. coli*	-	-	-	-
	S. aureus	-	-	-	-

Preliminary Phytochemical screening of HEUGL

The HEUGL was subjected to qualitative testing as per the procedure given in standard references[19,20]. The group of compounds identified in HEUGL are given in table 2.

Table 2: Phytoconstituents of the HECCL

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 HEUGL revealed presence of alkaloids, flavonoids, glycosides, sterols, tannins, and phenolic compounds. The IR,1H-NMR, and mass spectral data of HDC was found to be in agreement with its structure. The characteristic 1H-NMR signal at 4.38 & 9.12 for NH_2 & NH, appearance of IR band at 3259 & 1695 cm^{-1} and m/z value at 254 supported the successful synthesis of HDC. These spectral values were also further confirmed based on the literary facts[176,177]. Studies correlates the mechanics' of spread of diseases or ailments at molecular level and molecular therapeutics or approaches to treat them[178-212]. Literary facts suggest use of plants in the treatment of various diseases and to possess strong antimicrobial potential. Facts suggests phytochemical to elicit strong antimicrobial activity attributed to their phenolic content[211]. Evidence reports *S. aureus* and *E. coli*, to cause periodontitis. Growing incidences of microbial resistance towards conventional antibiotics raises the demand for evaluation of antimicrobials[5-8]. Evidence suggests use of *Uncaria gambir* in the treatment of various diseases and to possess strong antimicrobial potential. 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 PAB (Staphylococcus aureus and Escherichia coli) using well diffusion method. HEUGL was prepared using hydroalcoholic extract 50%. Prepared HEUGL was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. HEUGL showed good inhibitory effect overgrowth of S. aureus and E. coli. On the other hand, the HDC was prepared by amination of ester of catechol, and when tested against PAB (Staphylococcus aureus and Escherichia coli) exhibited high inhibitory potential study revealed that HDC possesses high potential when compared with HEUGL. Present study results were also supported my other study[211], however, further preclinical, and clinical studies are required to further support the antibacterial potential of HDC in periodontitis.

CONCLUSION

Based on present study over inhibitory potential of HDC and HEUGL against PAB, it is here by concluded that HDC possess high antibacterial potential against PAB especially *S. aureus* and *E. coli*. Present study recommends that highly potent HDC 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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