Potential of New Ferulic Acid analogue and *Paeonia lactiflora* Roots Extract and Cellulitis Triggering Pathogens

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ABSTRACT
Facts over *Escherichia coli* and *Staphylococcus aureus* ability to trigger cellulitis, and antimicrobial potential of *Paeonia lactiflora* plant motivated present study to compare the inhibitory potential of ferulic acid analogue (FAA) and *Paeonia lactiflora* root extract (PLRE) against cellulitis triggering bacteria (CTB). Current study involved synthesis of FAA and preparation of PLRE. The PD was characterized using ATR-IR, 1H-NMR and Mass spectrometric data. Both FAA and PLRE were further investigated for their antibacterial activity against CTB namely: *Escherichia coli* and *Staphylococcus aureus*. Among two, the FAA exhibited high antibacterial activity when compared with PLRE. Based onsss the results, present study concludes that FAA possess high antimicrobial potential against CTB and recommends that FAA should be further investigated to support its clinical significance.

KEYWORDS: Cellulitis, ferulic acid, comparison, extract, and antibacterial

INTRODUCTION

Cellulitis may develop due to involvement of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*)[1]. The human microbiome is known to possess 1:1 of bacteria and human cells, such that a small disturbance in this ratio may activate the mastitis triggering bacteria[2,3]. Long-term administration of conventional antibiotics against various infections may lead to mortality[4]. This problem can be handled using two therapeutic approaches, such as: use of synthetic or phytoproducts. Research suggests phenols and their derivatives to possess high antimicrobial potential[5-7], also evidence suggests that plants products and extracts act as effective antimicrobial therapy[8-13], so can be used for cellulitis triggering bacteria (CTB). Phytotherapy is a traditional economical approach for the treatment of various diseases and infections[14-16]. Since it elicits numerous biological activities, so used in wide range of diseases and ailments such as antiinflammatory [17,18], in obsessive compulsive disorder[19], digestant[20], antioxidant [21-25], antiurolithaitic[26], nephtoprotective[27,28], antiarrhythmic[29], antidepressant[30], anthelmintic[31], kidney disorders[32], cardiovascular disorders[33], antihyperlipidemic[34], diabetes[35-38], immunity booster[39], periodontitis[40-42], antidiarrhoeal[43], anticancer [44-53], hepato protective [54-65] and other pharmacological activities[66-69]. Many research highlighted increase in biological activity of plants together when used with nanotechnology[70-85].

Facts suggest several synthetic compounds to possess strong antimicrobial activity[86-105], due to which several plants product have been developed[106-122] and patented because of their significant biological activities[123-137]. Earlier research described the phyto-screening[176-179], isolation and characterization of several phytochemicals[138-175]. Hence, current study was aimed to determine the inhibitory potential of ferulic acid analogue (FAA) and *Paeonia lactiflora* root extract (PLRE) against cellulitis triggering bacteria (CTB).

MATERIAL AND METHODS

Materials

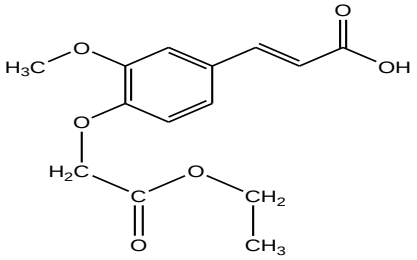
Melting points of newly synthesized compounds were determined using Thomas Hoover apparatus. IR spectra were recorded ATR-IR, Perkin Elmer, 1H-NMR on Bruker, DPX 300 and mass spectra on MASPEC (MSW/9629). Purity of synthesized compound was checked by TLC aluminium sheets – silica gel 60 F254 (0.2 mm). 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 *Paeonia lactiflora* root extract (PLRE) was prepared as per the standard protocol[8]. Briefly, *Paeonia lactiflora* roots 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 PLRE.
The obtained PLRE was stored at 4°C in refrigerator for further evaluation of its antimicrobial activity against CTB.

Procedure for the synthesis of ferulic acid analogue (FAA)

The synthesis of PD was done as per the standard protocol with slight modifications[86-105]. Briefly, the ferulic acid was refluxed with equimolar concentration of ethylchloroacetate for 16 hours. The obtained product was extracted with ether and purified.



Response of FAA and PLRE against CTB

Preparation of bacterial culture

Bacterial strains of *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. 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[12,13].

Well Diffusion Method

The inhibitory potential of the prepared PLRE and FAA against CTB was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications[12-13]. 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 PLRE and FAA were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for PLRE, FAA, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the PLRE and FAA. 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 PLRE

The PLRE 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[176-179].

RESULTS

Synthesis of PD

Pale yellow liquid; Yield 84%; ATR-IR: 3037, 2925, 1720, 1795 cm^{-1}; ^{1}H-NMR δ (ppm): 1.30

(3H, t, CH_3), 2.56 (3H, s, CH_3), 4.15 (2H, q, O-CH_2), 4.92 (2H, s, O-CH_2), 6.61-6.75 (4H, m, Ar-H), 639 (1H, d, =CH), 7.63(1H, d, =CH)), 10.23 (1H, s, OH); MS: m/z: 280 (M^+).

Response of PLRE and FAA against CTB

In present study, the prepared PLRE and FAA, were evaluated for their inhibitory potential against CTB such as *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 PLRE and FAA

Compound	Microorganism	Zone of inhibition			
		Reading 1	Reading 2	Reading 3	Average Value
PLRE	*E. coli*	12	12	12	12
	S. aureus	15	15	15	15
FAA	*E. coli*	23	23	23	23
	S. aureus	22	22	22	22
Streptomycin	*E. coli*	24	24	24	24
	S. aureus	25	25	25	25
Tween 80	*E. coli*	-	-	-	-
	S. aureus	-	-	-	-

Preliminary Phytochemical screening of PLRE

The PLRE was subjected to qualitative testing as per the procedure given in standard references[176-179]. The group of compounds identified in PLRE are given in table 2.

Table 2: Phytoconstituents of the PLRE

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 PLRE revealed presence of alkaloids, flavonoids, glycosides, sterols, tannins, and phenolic compounds. The IR,1H-NMR, and mass spectral data of FAA was found to be in agreement with its structure. The characteristic 1H-NMR signal at 1.30 & 4.15, appearance of IR band at 1795 cm^{-1} and m/z value at 280 supported the successful synthesis of FAA. These spectral values were also further confirmed based on the literary facts[180,181]. Research correlates the mechanics' of spread of diseases or ailments at molecular level and molecular therapeutics or approaches to treat them[182-214]. Evidence reports *S. aureus* and *E. coli*, to trigger microbial resistance towards conventional antibiotics raises the demand for evaluation of antimicrobials[4-7]. Facts suggests phytochemical to elicit strong antimicrobial activity attributed to their phenolic content[215-217]. Reports suggests use of *Paeonia lactiflora* in the treatment of various diseases and to possess strong antimicrobial potential. As per the literature available over different parts of *Paeonia lactiflora* plant and yet much more must be explored for this plant. Hence, investigators of present study planned to evaluate the in-vitro inhibition potential of *Paeonia lactiflora* root extract against CTB (*Staphylococcus aureus* and *Escherichia coli*) using well diffusion method. The PLRE was prepared using hydroalcoholic extract 50%. The prepared PLRE was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. The PLRE showed good inhibitory effect overgrowth of *S. aureus* and *E. coli*. On the other hand, the FAA was prepared by esterification of ferulic acid, and when tested against CTB (*S. aureus* and *E. coli*) exhibited high inhibitory potential study revealed that synthetic derivative (FAA) possesses high potential when compared with PLRE. However, further preclinical, and clinical studies are required to further support the antimicrobial potential of FAA.

CONCLUSION

The results of the present study over inhibitory potential of FAA and PLRE against CTB, it is here by concluded that synthetic derivative

FAC possess high antimicrobial potential against CTB especially *S. aureus* and *E. coli*. Present study recommends that highly potent FAA 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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