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RESPONSE OF PAEONIA LACTIFLORA LEAVES EXTRACT AND HYDROXYACETOPHENONE DERIVATIVE AGAINST PATHOGENS THAT TRIGGERS ACUTE SEPTIC ARTHRITIS

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Abstract

Evidence over ability of *Escherichia coli* and *Staphylococcus aureus* to cause acute septic arthritis (ASA), and antibacterial potential of *Paeonia lactiflora* plant motivated present study to compare the inhibitory potential of hydroxyacetophenone derivative (HAD) and *Paeonia lactiflora* leaves extract (PLLE) against ASA triggering bacteria (ATB). Current study involved synthesis of HAD and preparation of PLLE. The HAD was characterized using ATR-IR, 1H-NMR and Mass spectrometric data. Both HAD and PLLE were further investigated for their antibacterial activity against ATB namely: *Escherichia coli* and *Staphylococcus aureus*. Among two, the HAD exhibited high antibacterial activity when compared with PLLE. Based on the results, present study concludes that HAD possess high antimicrobial potential against ATB and recommends that HAD should be further investigated to support its clinical significance.

Keywords: Acute septic arthritis, hydroxyacetophenone, Comparison, extract, and antibacterial

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INTRODUCTION

Acute septic arthritis (ASA) may develop due to direct introduction, or extension from a contiguous focus of infection. Pathogenesis of acute septic arthritis is multifactorial and depends upon interaction of host immune response and the adherence factors, toxins, and immunoavoidance strategies of the invading pathogen. Facts suggest involvement of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in acute septic arthritis[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 ASA triggering pathogens[2,3]. Long-term administration of conventional antibiotics against various infections lead to potential mortality risk[4]. This problem can be handled using two therapeutic approaches, such as: use of synthetic or phytoproducts. Findings suggests phenols and their derivatives to possess high antimicrobial potential[5-7]. Evidence suggests that plants products and extracts act as effective antimicrobial therapy[8-13], therefor these can be used for the problem of ASA. Phytotherapy is a traditional economical approach for the treatment of various diseases[14-16]. Plants are known to elicit numerous biological activities so used in wide range of diseases and ailments such as digestant[17], in obsessive compulsive disorder[18], antiinflammatory[19,20], antiarrhythmic[21], antioxidant[22-26], antidepressant[27], anthelmintic[27], anthelmintic[28], kidney disorders[29], cardiovascular disorders[30], diabetes[31-34], antihyperlipidemic[35], periodontitis[36-38], immunity booster[39], antidiarrhoeal[40], antiurolithaitic[41], hepatoprotective[42-53], nephtoprotective[54,55], anticancer[56-65]and other pharmacological activities[66-69]. A large volume of research revealed increase in biological activity of plants when used with nanotechnology[70-85]. Evidence suggests several synthetic moieties that possess strong antimicrobial activity[86-105], also numerous plants product have developed[106-122], and patented attributed to their higher biological potential[123-137]. Literature describes isolation of several phytochemicals[138-175], their phyto-screening[176-179], and characterization. Hence, present study was aimed to determine the inhibitory potential of hydroxyacetophenone derivative (HAD) and *Paeonia lactiflora* leaves extract (PLLE) against ASA triggering bacteria (ATB).

MATERIAL AND METHODS

Materials

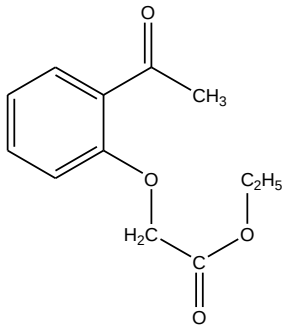
Melting points of newly synthesized compounds were determined using Thomas Hoover apparatus. IR spectra were recorded ATR-IR, Perkin Elmer, 1H-NMR on Bruker, DPX 300 and mass spectra on MASPEC (MSW/9629). Purity of synthesized compound was checked by TLC aluminium sheets – silica gel 60 F254 (0.2 mm).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

Procedure for the synthesis of hydroxyacetophenone derivative (HAD)

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



Response of HAD and PLLE against ATB

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 PLLE and HAD against ATB 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 PLLE and HAD were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for PLLE, HAD, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the PLLE and HAD. 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 PLLE

The PLLE 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 HAD

Pale yellow liquid; Yield 76%; ATR-IR: 3045, 2926, 1715, 1798 cm^{-1}; ^{1}H-NMR δ (ppm): 1.32 (3H, t, CH_3), 2.56 (3H, s, CH_3), 4.15 (2H, q, O-CH_2), 4.91 (2H, s, O-CH_2), 6.81-7.35 (4H, m, Ar-H); MS: m/z: 222 (M^+).

Response of PLLE and HAD against ATB

In present study, the prepared PLLE and HAD, were evaluated for their inhibitory potential against ATB 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 PLLE and HAD

Compound	Microorganism	Zone of inhibition			
		Reading 1	Reading 2	Reading 3	Average Value
PLLE	*E. coli*	10	10	10	10
	S. aureus	14	14	14	14
HAD	*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 PLLE

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

Table 2: Phytoconstituents of the PLLE

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 PLLE revealed presence of alkaloids, flavonoids, glycosides, sterols, tannins, and phenolic compounds. The IR,1H-NMR, and mass spectral data of HAD was found to be in agreement with its structure. The characteristic 1H-NMR signal at 1.32 & 4.15, appearance of IR band at

1798 cm^{-1} and m/z value at 222 supported the successful synthesis of HAD. 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* leaves extract against ATB (*Staphylococcus aureus* and *Escherichia coli*) using well diffusion method. The PLLE was prepared using hydroalcoholic extract 50%. The prepared PLLE was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. The PLLE showed good inhibitory effect overgrowth of *S. aureus* and *E. coli*. On the other hand, the HAD was prepared by esterification of hydroxyacetophenone, and when tested against ATB (*S. aureus* and *E. coli*) exhibited high inhibitory potential study revealed that synthetic derivative (HAD) possesses high potential when compared with PLLE. However, further preclinical, and clinical studies are required to further support the antimicrobial potential of HAD.

CONCLUSION

The results of the present study over inhibitory potential of HAD and PLLE against ATB, it is here by concluded that synthetic derivative HAD possess high antimicrobial potential against ATB especially *S. aureus* and *E. coli*. Present study recommends that highly potent HAD 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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