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RESPONSE OF NEW GALLIC ACID DERIVATIVE AND PUNICA GRANATUM SEEDS EXTRACT AGAINST MENINGITIS TRIGGERING PATHOGENS

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Abstract

Some atypical bacteria may cause upper respiratory tract infection and may result in meningitis by traveling via bloodstream to brain. Facts over *S. aureus* and *E. coli* to cause meningitis, antimicrobial potential of *Punica granatum* plant and gallic acid intended current study to compare the antibacterial response of new gallic acid derivative (GAD) and *Punica granatum* seeds extract (PGSE) against meningitis triggering bacteria (MTB). Present study involved synthesis of GAD and preparation of PGSE. The GAD was characterized using ATR-IR, ¹H-NMR and Mass spectrometric data. Both PGSE and GAD were tested for inhibitory potential against MTB, namely: *S. aureus* and *E. coli*. Amid both, the GAD exhibited high inhibitory potential against MTB, when compared with PGSE. Based on the results present study concludes that GAD possess high inhibition potential against MTB and recommends that GAD should be further tested to support its clinical significance.

Keywords: Meningitis, *Punica granatum*, seeds, extract, meningitis, and antibacterial

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INTRODUCTION

Meningitis due to bacteria is associated with high rates of mortality. The bacterial meningitis (BM) causing bacteria such as *S. aureus* and *E. coli* should be considered while initiating the empirical antimicrobial therapy for BM¹. Human microbiota comprises bacteria and human cells in almost equal ratio, a small disturbance in such ratio may activate the meningitis triggering bacteria (MTB)². Evidence suggests role of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in bacterial meningitis³. Long-term administration of commercially available antibiotics against various infections manifests in multiple drug resistance and risk of high mortality⁴⁻⁶. For such problem, one may use synthetic or phytoproduct therapy. Literary facts support plants products as an effective antibacterial agent⁷⁻¹⁵, so they can be used to combat MTB. Herbal treatment is an economical approach for the treatment of various diseases¹⁶⁻¹⁸. Plants are known to exhibit numerous biological activities, so can be used in wide range of infections, disorders and diseases like: antiarrhythmic¹⁹, obsessive compulsive disorder²⁰, digestant²¹, antiinflammatory²²⁻²³, hepatoprotective²⁴⁻³⁵, diabetes³⁶⁻³⁹, antioxidant⁴⁰⁻⁴⁴, periodontitis⁴⁵⁻⁴⁷, anthelmintic⁴⁸, antidepressant⁴⁹, nephroprotective^{49,50}, antiurolithaitic⁵¹, kidney disorders⁵², cardiovascular disorders⁵³, antihyperlipidemic⁵⁴, immunity booster⁵⁵, anticancer⁵⁶⁻⁶⁵, antidiarrhoeal⁶⁶, digestant⁶⁷, and other pharmacological activities⁶⁸⁻⁷¹. Many studies revealed increase in biological potential of plants when combined with nanotechnology⁷²⁻⁸⁵. Studies reveals several synthetic moieties to possess high antimicrobial potential⁸⁶⁻¹⁰⁵. Numerous plants product has been developed¹⁰⁶⁻¹²², and patented due to high biological potential¹²³⁻¹³⁷. Studies described isolation of various phytochemicals¹³⁸⁻¹⁷⁵, their phyto-screening and characterization¹⁷⁵⁻¹⁸¹. Therefore, present study was intended to determine the antimicrobial potential of *Punica granatum* seeds extract (PGSE) and new gallic acid derivative against MTB.

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,

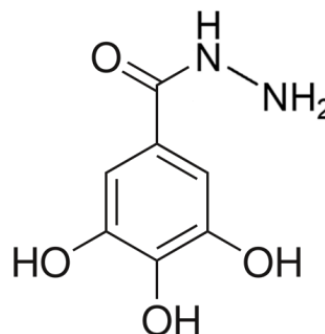
Malaysia. Chemicals, and solvents were procured from the SD Fine, Sigma-Aldrich, and Merck Ltd.

Preparation of Plant Extract

The PGSE was prepared as per the standard procedure in the literature¹⁵⁻²⁵. Briefly, *Punica granatum* seeds free of decay were collected from the market of Sungai Petani, Kedah state, Malaysia and washed with tap water, followed by air drying, 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 PGSE. The obtained PGSE was stored at 4°C in refrigerator for further evaluation of its inhibitory activity against MTB.

Procedure for the synthesis of GAD

The synthesis of new GAD was done as per the standard protocol with slight modifications¹⁴⁰⁻¹⁴³. Briefly, equimolar concentration of Gallic acid and hydrazine hydrate was refluxed for 4 hours. The crystals obtained were separated, washed with cold water, dried, and recrystallized from ethanol.



Response of PGSE and GAD against MTB

Preparation of bacterial culture

Bacterial strains of *S. aureus* and *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^{11,12}.

Well Diffusion Method

The inhibitory potential of the prepared PGSE and GAD against MTB was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications¹³²⁻¹³⁴. 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 PGSE and GAD were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for PGSE, GAD, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the PGSE and GAD. 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 PGSE

The PGSE 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 GAD

Pale yellow crystals; Yield 74%; mp 162°C; ATR-IR: 3045, 1698, and 1259 cm⁻¹; ¹H-NMR δ (ppm): 4.37 (2H, brs, NH₂), 4.8 (3H, brs, OH), 7.3 (2H, s, Ar-H), 9.52 (1H, brs, NH); MS: m/z: 172 (M⁺)

Response of PGSE and GAD against MTB

In present study, the prepared PGSE and evaluated for their inhibitory potential against MTB such as *S. aureus* and *E. coli* using agar well diffusion for measurement of zone of inhibition. The prepared PGSE and GAD 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 PGSE and GAD

Compound	Microorganism	Zone of inhibition			Average Value
		Reading 1	Reading 2	Reading 3	
PGSE	<i>E. coli</i>	12	12	12	12
	<i>S. aureus</i>	15	15	15	12
GAD	<i>E. coli</i>	22	22	22	22
	<i>S. aureus</i>	20	20	20	20
Streptomycin	<i>E. coli</i>	24	24	24	24
	<i>S. aureus</i>	25	25	25	25
Tween 80	<i>E. coli</i>	-	-	-	-
	<i>S. aureus</i>	-	-	-	-

Preliminary Phytochemical screening of PGSE

The PGSE was subjected to qualitative testing as per the procedure given in standard references¹⁸⁻²⁰. The group of compounds identified in PGSE are given in table 2.

Table 2: Phytoconstituents of the SSE

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 PGSE revealed presence of alkaloids,

flavonoids, glycosides, sterols, tannins, proteins and phenolic compounds. The IR, ¹H-NMR, and mass spectral data of GAD was found to be in agreement with its structure. The characteristic ¹H-NMR signal at 4.37 & 9.52, appearance of IR band at 1698 cm⁻¹ and m/z value at 172 supported the successful synthesis of GAD. These spectral values were also further confirmed based on the literary facts¹⁸⁰⁻¹⁸¹. Evidence reports *S. aureus* and *E. coli*, to trigger meningitis. The growing incidences of microbial resistance towards conventional antibiotics raises the demand for evaluation of new antimicrobials⁷⁻¹⁰. Research correlates the mechanics' of spread of diseases or ailments at molecular level and molecular therapeutics or approaches to treat them¹⁸¹⁻²¹⁵. Reports suggests use of *Cinnamomum iners* in the treatment of various diseases and to possess strong antimicrobial potential. Facts suggests phytochemical to elicit strong antimicrobial activity attributed to their phenolic content²¹⁶⁻²¹⁷.

Reports suggests use of *Punica granatum* plant in the treatment of various diseases and to possess strong antimicrobial potential. As per the literature available over different parts of *Punica granatum* 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 *Punica granatum* seeds extract against MTB (*S. aureus* and *E. coli*) using well diffusion method. The PGSE was prepared using hydroalcoholic extract 50%. The prepared PGSE was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. The PGSE showed good inhibitory effect overgrowth of *S. aureus* and *E. coli*. On the other hand, the GAD was prepared by amination of gallic acid, and when tested against MTB (*S. aureus* and *E. coli*) exhibited high inhibitory potential study revealed that GAD possesses high potential when compared with PGSE. The results of the present study were also supported by many other studies¹⁵⁵⁻¹⁸². However, further preclinical, and clinical studies are required to further support the antimicrobial potential of GAD.

CONCLUSION

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