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REGULAR ARTICLE

ANTIBACTERIAL EFFECT OF *PERSICARIA THUNBERGII* ON *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

With the discovery of various antibiotic resistant bacteria, evaluations of antimicrobial activities of natural compounds have been preceded on antibiotic susceptible and resistant microorganisms. Several types of natural compounds have been reported to have similar effects on target microorganisms as compared to the widely used antibiotics. *Persicaria thunbergii* (Polygonaceae) has been known to have anti-tumoral, anti-angiogenesis, anti-oxidation and anti-inflammation functions. In this study, aerial parts of *P. thunbergii* were extracted using methanol, chloroform, and ethyl acetate to identify possible anti-bacterial effects. Agar disk diffusion method and time-kill assay were done to evaluate the antibacterial effect of *P. thunbergii* extracts. Two extracts ethyl acetate (EAE), and chloroform (CFE) were tested against *Staphylococcus aureus*. As a result, the extract from CFE and EAE showed antibacterial effect against *S. aureus*. The extract EAE showed the strongest inhibition effect compared to CFE. These results demonstrate that the EAE extract which originated from *P. thunbergii* can probably play a role as an antibacterial agent.

Keywords: Antibacterial effect, Disk diffusion, *Persicaria thunbergii*, *Staphylococcus aureus*, time-kill assay

INTRODUCTION

Development of antibiotic resistance in bacteria is a critical issue in the controlling of infectious diseases [1, 2]. Therefore, antibacterial agents are needed to be found and apply to control multi-drugs resistant bacteria [3]. There has been growing interests to find antimicrobial compounds with different mechanism of action from natural resources such as plant extracts as an alternative approach to deal with this problem [4]. In recent years, the *Staphylococcus aureus* has been identified as a resilient and resistant pathogen [1, 4]. The hospitals outbreak of multi-drug resistant *S. aureus* has been reported worldwide. Only ~ 20 % of the *S. aureus* strains remain sensitive to penicillin [4], ~ 60 % of the *S. aureus* clinical isolated strains in United State of America shows resistance to methicillin [5]. The failure of existing antibiotics in controlling the *S. aureus* infections has increased interest in finding an alternative treatment. The natural resource such as medicinal plants plays a significant role in primary healthcare system. The plants extract contains different phytochemicals such as alkaloids, steroids, flavonoids, proteins, tannins, phenolic compounds; and secondary metabolites which plays a defensive work against foreign infectious agents to protect plant.

The *Persicaria thunbergii* is an herb with 30-100 cm height and long petioles with wings; belongs to family Polygonaceae. This plant is famous in Korea and China for folk medicinal uses in curing rheumatism, measles and hemorrhage. *P. thunbergii* is a medicinal plant; recently significant activities reported in biomedical applications such as, anti-tumoral [6], isorhamnetin induced-apoptosis [7], anti-oxidation [8], anti-inflammation [9], anti-angiogenesis [10], and flavonoids from *P. thunbergii* reported as a acetylcholinesterase inhibitor [11]. However, to date, there is no data on antibacterial activity of *P. thunbergii* on pathogenic bacteria. Thus, the present study aimed to determine the antibacterial activity of *P. thunbergii* different extract on *Staphylococcus aureus* bacteria.

MATERIALS AND METHODS

Materials

The aerial part of *P. thunbergii* was collected from Yangsa, Gyeongnam in Korea. Mueller-Hinton Agar (MHA) and Brain Heart Infusion (BHI) broth were obtained from BD diagnostic, Le Pont De Claix, France. *Staphylococcus aureus* ATCC6538P was procured from Korean Culture

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Plant material extraction and fractionation

Sample extract preparation and separation process perform as described previously with minor modifications [9]. In brief, the aerial part of *P. thunbergii* was dried at room temperature and powder was prepared using electric grinder. The prepared powder was soaked in methanol at room temperature, after 4 w residue separated by filtration. The filtrate was concentrated in vacuum condition. After that, methanol extract was fractioned with chloroform using separation funnel. Consequently, ethyl acetate extracts were fractioned using the same method. Each extracts were adsorbed on the silica gel and followed separation by automated flash chromatography system (MPLC; Combifresh-RF. ISCO) and samples dried under vacuum condition.

Disk diffusion assay

This method was performed as per CLSI guideline description [12]. *S. aureus* were separately inoculated in tube containing 5 ml BHI broth, and incubated at 37 °C for 4 h in rotary shaking incubator. After incubation, 100 µl of each of the bacterial suspension approximately 10^5 CFU. ml^{-1} were spreaded over the surface of MHA plate using sterile glass spreader. Paper disk (8 mm diameter), were dropped with 10 µg penicillin and different concentrations (1, 3, and 5 mg) of *P. thunbergii* ethyl acetate extract (EAE) and chloroform extracts (CFE) separately. After that, paper disk placed in four corners on the surface of MHA plates inoculated with bacteria and incubated at 37 °C for 17 h. After incubation diameter of bacterial growth inhibition surrounding the paper disk called zone of inhibition (ZOI) were measured in mm.

Bacterial viability test

The *S. aureus* suspension was prepared as mentioned in previous test. Six tubes with 5 ml BHI broth were prepared and inoculated with *S. aureus* suspension. First tube was set as a control, and in second tube 20 µl of dimethylsulfoxide (DMSO) was added. After which 20 µl of EAE and CFE with a concentration 400 and 800 µg. ml^{-1} were added, respectively to each of the remaining tubes. All the tubes were incubated at 37 °C for 17 h. After that, serial dilutions were prepared from 10^{-1} to 10^{-7}. Then, 100 µl of 10^{-6} and 10^{-7} dilutions were separately spreaded over the surface of BHI agar in duplicates. All plates after spreading were incubated at 37 °C for 17 h. The former colonies on the agar plates were counted to determined which samples were to be subjected to time kill assay.

Time kill assay

Seven tubes with 5 ml BHI broth were prepared. All tubes were inoculated with *S. aureus*. One tube was set as a control, and in another tube 20 µl of dimethylsulfoxide (DMSO) was added. After which 20 µl of EAE and CFE with a concentration 400 µg. ml^{-1} were added to 2 tubes, respectively. The remaining 3 tubes were added with a concentration 400 µg. ml^{-1} of the EAE fractions (EAE1, EAE2, and EAE3) separately. All tubes were incubated at 37°C and optical density (OD) at 620 nm was observed at interval of every 4 h up to 24 h.

RESULTS AND DISCUSSION

The *P. thunbergii* extracts were screened for antibacterial susceptibility by disk diffusion method against *S. aureus*, the concentration dependent ZOI were observed against *S. aureus*. Fig.1 (a) indicates ZOI around disk contains (1) Penicillin10 µg, (2, 3, and 4) 1, 3, and 5 mg of EAE respectively, likewise, Fig.1 (b) indicates ZOI around disk contains (1) Penicillin10 µg (2, 3, and 4), 1, 3, and 5 mg of CFE, respectively. Penicillin shows ZOI (14 mm) smaller than EAE, and CFE. ZOI for EAE were recorded 17, 22, and 24 mm at concentration per disk was 1, 5 and 5 mg, respectively. While, ZOI for CFE were observed 22, 23, and 24 mm at 1, 3 and 5 mg per disk, respectively.

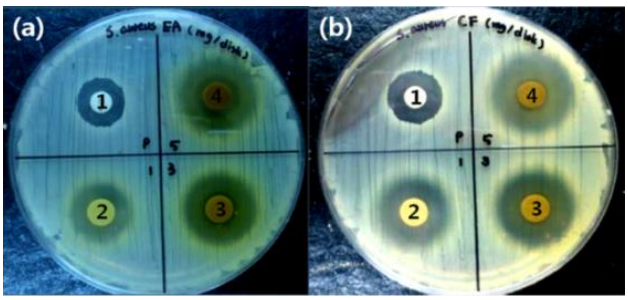

Fig. 1: Zone of inhibition of *Persicaria thunbergii* extracts (a) Ethyl acetate extract and (b) Chloroform extract on *Staphylococcus aureus*

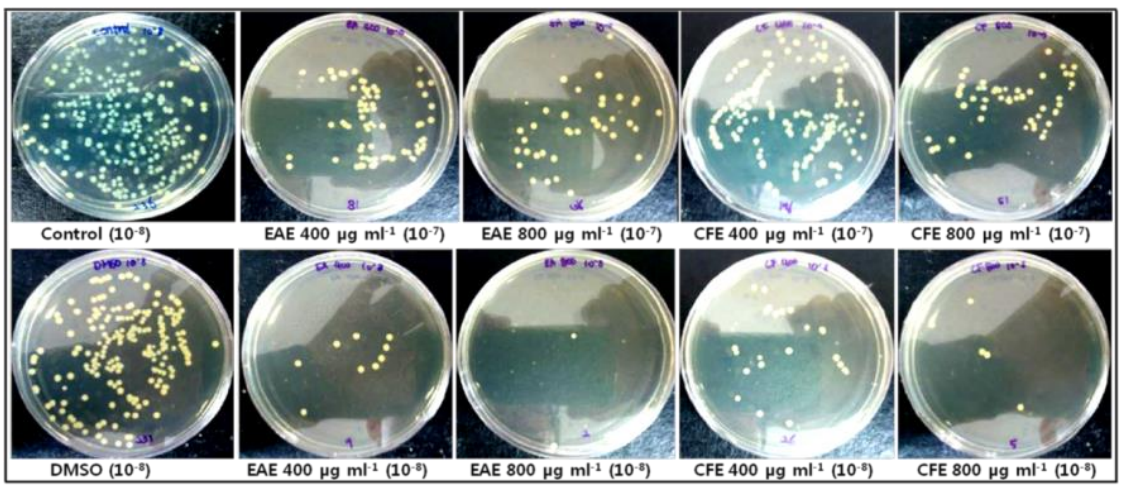

Fig. 2: *Persicaria thunbergii* extracts exhibits concentration dependent antibacterial susceptibility against *Staphylococcus aureus*. (DMSO-Dimethylsulfoxide, EAE-Ethyl acetate extract, CFE-Chloroform extract)

The bacterial viability test was performed against *S. aureus* by spread plate method. The *P. thunbergii* extracts were observed concentration dependent antibacterial activity. EAE and CFE treated bacterial suspension tubes, after incubation were subjected to serial dilution and spreaded on agar surface to develop colonies for determining bactericidal effect. Fig. 2 indicates, Untreated (control) and DMSO containing bacterial suspension (10^{-8}), EAE, and CFE treated bacterial suspension (10^{-7}, 10^{-8}) at 400 and 500 µg. ml^{-1} concentrations were developed colonies. The number of colonies counted (table 1) and indicated the *S. aureus* were concentration dependent susceptible; EAE shows strong bactericidal effect compared to CFE at both concentrations.

After *P. thunbergii* extracts treated bacterial viability check, 400 µg. ml^{-1} EAE and CFE were used to determine the time dependent bactericidal effect. Time kill assay were performed by spectroscopic method. Fig.3 indicates the untreated (control) and DMSO containing bacterial suspension shows time dependent increased absorbance at 600 nm indicates multiplication of bacteria, comparatively EAE and CFE shows less absorbance. The EAE shows more bacteria killing potential than CFE.

The *P. thunbergii* aerial part contains flavonoids and flavonoids glycosides such as isorhamnetin, quercetin, and shows potential anticancer, antitumor and anti-oxidant activity without any toxic effect on human cells [6, 9-11]. Flavonoids are the good source as an antibacterial agent [13], and due to non-toxic nature and natural resource *P. thunbergii* extract can be useful as a new antibacterial agent. Other members of genus *Persicaria*, such as *P. odorata* [14] and *P. minor* (Huds.) [15] were reported for their potential antimicrobial activity. This is the first reporting for antibacterial potential of *P. thunbergii* against *S. aureus*. Overall, *P. thunbergii* EAE has good antibacterial activity against *S. aureus*, the phytocomposition and exact mechanism behind *S. aureus* growth inhibition needs more detail studies.

CONCLUSION

In conclusion, plant extracts have great potential as antimicrobial agent. Thus, it can be used in treating of infectious diseases caused by pathogenic bacteria. In this study, extracts of *P. thunbergii* was found to exhibit antibacterial effects on *S. aureus*. However the mechanism of antibacterial properties of *P. thunbergii* extract is still unknown. These findings suggest that the extract from *P. thunbergii* is can be useful in healthcare and medicines as an antibacterial agent.

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CONFLICT OF INTERESTS

No conflict of interest declared

Table 1: Bacterial viability test. *P. thunbergii* EAE and CFE shows concentration dependent *S. aureus* inhibition. (DMSO-Dimethylsulfoxide, EAE-Ethyl acetate extract, CFE-Chloroform extract, and TNTC-Too numerous to count)

Dilution	Control	DMSO	EAE 400 µg. ml^{-1}	EAE 800 µg. ml^{-1}	CFE 400 µg. ml^{-1}	CFE 800 µg. ml^{-1}
10^{-7}	TNTC	TNTC	81	36	196	51
10^{-8}	236	231	9	2	26	5

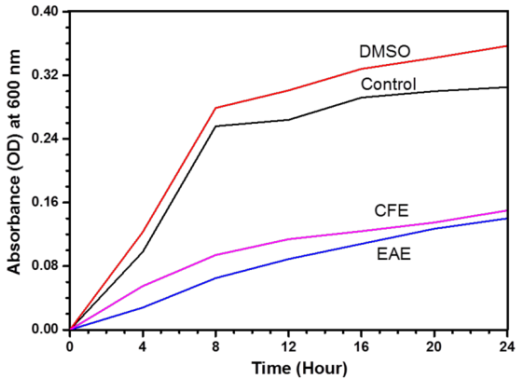

Fig. 3: Time kill assay. *Persicaria thunbergii* EAE and CFE shows time dependent *Staphylococcus aureus* inhibition. (DMSO-Dimethylsulfoxide, EAE-Ethyl acetate extract, CFE-Chloroform extract, and TNTC-Too numerous to count)

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