Received: 26 April 2022 | Revised: 07 June 2022 | Accepted: 28 June 2022
DOI: 10.22034/ecc.2022.344320.1475
Eurasian Chemical Communications
SAMI

FULL PAPER

Antibacterial activity of *Cassia angustifolia.* Vahl (*Sinameki*) leaf extract against some pathogenic bacteria

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Cassia angustifolia. Vahl *(Sinameki)* is one of the best-known medicinal herbs for the treatment of constipation in Iraq and many Arabic countries and it has long been used as a medicinal agent. In the present study, the antibacterial potential of *Sinameki* and its synergistic effect with antibiotics including tetracycline and amoxicillin were investigated at various concentrations (50, 100, and 150 mg/mL) against three pathogenic bacterial strains including *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*). A dilution method was used to determine the MIC (minimum inhibitory concentration) of the plant leaf extract against *E. coli*, *S. aureus* and *P. aeruginosa*. The synergistic effect between the extract and antibiotics was evaluated using a diffusion method. *Sinameki* (methanol extract) showed a potent inhibitory activity against all the tested bacteria, while *Sinameki* (methanol extract) revealed lower inhibitory activity against all tested bacteria compared to the negative control. The highest inhibitory activity of *Sinameki* (methanol extract) was confirmed against *E. coli*, *S. aureus* and *P. aeruginosa* with an inhibition zone of 8, 5, and 4 mm, respectively. The combination of *Sinameki* extract with antibiotics including tetracycline and amoxicillin has resulted in increasing the antibacterial activity. The findings are very important for further study of the *Sinameki* as an alternative herbal medicine leading to cure diseases caused by microbial pathogens.

KEYWORDS

Cassia angustifolia. Vahl; antimicrobial; drug discovery; herbal medicine; extract.

Introduction

Antimicrobials are likely one of the most effective kinds of chemotherapy in medical history and used to prevent and treat infections caused by bacteria. Antibiotics play an important role to compact, fight, and end bacterial resistance [1]. Pathogenic microbes that are resistant to many or all currently available antibiotics are a major public health problem, particularly in clinical settings. In its Global Risks 2013 report, the World Economic Forum recognized antibiotic resistance as one of the most serious dangers to human health [2]. Infectious diseases have long been a source of concern [3]. Every year, about 2 millions in North Americans acquire diseases linked to antibiotic resistance, resulting in 23,000 deaths [4]. Nearly 700,000 cases of antibiotic-resistant illnesses occur in Europe each year, resulting in over 33,000 deaths [5] at a cost of over €1.5 billion [6]. Despite a 36% rise in human antibiotic use from 2000 to 2010, infectious diseases still account for nearly 20% of deaths worldwide today [7,8]. Medicinal plants or known as medicinal herbs

have been attracted much of interest due to their therapeutic potentials which can be used for the treatment of infectious diseases [9]. Herbal medicine are considered to be a source of a various secondary metabolites used as potential drugs [10-12] and cosmetics [13]. *Sinameki* is a Turkish name of a family of *Cassia angustifolia*. Vahl [14]. *Sinameki* leaf contains mostly anthracene and anthraquinone derivatives such as Rhein as antibiotic agent, Sennoside A as laxative agent, and *β*-Sitosterol as anticancer agent (Figure 1) [15] and has many therapeutic properties for treatment of constipation, leprosy, jaundice, typhoid fever, cancer, leukoderma, circulation troubles, and eczema [16-20]. The aim of this study to find the potent bioactive materials either using synthesis method [21-48] or by the study of therapeutic properties of traditional medicinal herbs [49]; therefore, we report the antibacterial activity and synergistic effect of *Sinameki* extract utilizing water and methanol.

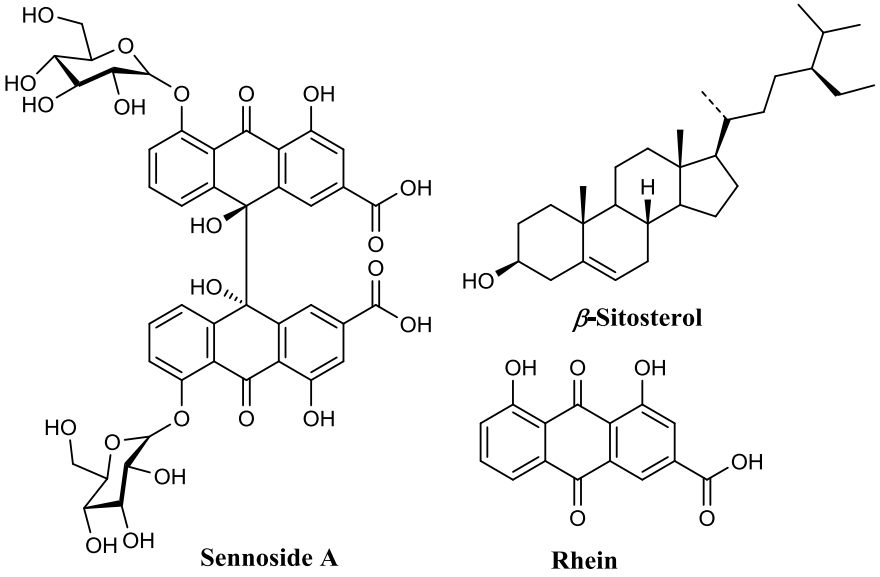

FIGURE 1 Bioactive molecules of *Cassia angustifolia.* Vahl

Material and methods

Collection of microorganism samples

Pathogenic antibiotic resistant bacteria were collected from medical laboratory. Brain heart infusion (BHI) Agar was used as for the cultivation of microorganisms including *E. coli, P. aeruginosa*, and *S. aureus*. Brain heart infusion (BHI) Agar was prepared by adding 15 g of agar to 1 liter of distilled water followed by heating to dissolve agar before dispensing into bottles or flasks and they were autoclaved for 15 minutes at 121 °C [49].

Preparation of the plant samples

Dried *Sinameki* leaf was collected from local Erbil Market and we prepared two extracts, the first one from water and the second from methanol.

Preparation of methanolic extract

200 mL of methanol (80%) was added at 45 °C for 7 hr to a 30 g of *Sinameki* leaf in the soxhlet system [49]. Subsequently, the extract was evaporated at 37 °C until completely evaporating. The extract is then collected, weighed, and dissolved in distilled water to obtain a 20% (w/v) and sterilized using 0.4 µm filtration paper in order to be free of microbial contamination.

Preparation of water extract

200 mL of distilled water was added to a 30 g of *Sinameki* powder followed by boiling the

solution for 7 hours. The extract was filtered, evaporated, weighed, and dissolved in distilled water resulting in obtaining 20% (w/v) followed by sterilizing sterilized using 0.4 µm filtration paper to get rid of the contaminated materials [49].

Antimicrobial susceptibility testing

The cultures have developed by transferring 5 mL of brain heart infusion broth from a cultivated lope into the incubator at 37 °C for 24 hours. Culture media was inoculated with activated bacterial growth. The sensitivity of bacteria to antibiotics of amoxicillin and tetracycline (obtained from Samarra Pharmaceutical Company-Iraq) were tested using the cup plate technique diffusion method by diluting of used antibiotics at various concentrations of 50, 100, and 150 µg/mL, and it was considered as a positive control. Twenty-seven amoxicillin tests were conducted including nine tests were conducted for each type of bacteria at different concentrations of 50, 100, and 150 mg and the same method has applied to tetracycline. The dishes were kept in the incubator for 24 hours at 37 °C and the inhibition areas of antibiotics were measured and compared to the inhibition areas of the used plant concentrations measured in millimeter [50].

Testing the effectiveness of Sinameki extract against pathogenic bacteria

Distilled water was added to *Sinameki* extract for preparation of various concentration of 50, 100, and 150 mg/mL. Cup plate technique method has employed using of a perforator to test the efficiency of plant extracts against bacteria which having holes of 6 mm on the culture media. 0.2 mL of each concentration was added to each hole while keeping a hole containing sterile distilled water as a negative control. Twenty-seven tests were performed for the water extract involving nine tests were conducted for each type of bacteria at different concentration of 50, 100, and 150 mg and the same method has been applied for alcoholic extract. The culture media plates were kept in the refrigerator for 4 hours for spreading the extract and the dishes were then incubated at 37 °C for 24 hours to dissipate the extract from each hole and sterile water was left as a negative control [49].

Minimum inhibitory concentration (MIC) determination

A series of half-dilutions were prepared from the last concentration of 200 mg/mL of the plant leaf extract in sterile test tubes of 6.25, 12.5, 25, 50, 100, and 200 mg/mL and Mueller Hinton broth medium was used to make the dilutions, then the tubes were inoculated with 0.1 mL (approximately $1.5X10^8$ CFU/mL) of the bacterial suspension according to the multiplication dilutions. As prepared previously, the test tubes were incubated at 37 °C for 24 hours and the results were determined in comparison with control model 1 which contains broth media inoculated with bacteria only, and control model 2 which contains broth media with plant extract without bacteria.

Synergism of antibiotics and Sinameki extracts

The bacterial samples were grown in Mueller Hinton broth at 37 °C and each bacterium was inoculated on the surface of the Mueller-Hinton agar plate after 4 hours. The absorbed antibiotic was then placed on the paper disk (diameter=5 mm) of each inoculated plate surface followed by adding 20 µL of plant extract to identify the effect of synergy between a 200 mg/mL plant extract and antibiotics. Nineteen tests were conducted for tetracycline including nine tests for the alcoholic extract and another nine tests for the aqueous extract at a specific concentration of tetracycline and a specific concentration of the plant extract as well as the same method was performed for amoxicillin. The plates were incubated at 37 °C for 24 hours and the clearing area diameters were measured.

Results and discussion

Tetracycline showed potent inhibitory activity against *E. coli*, *P. aeruginosa* and *S. aureus* at the concentration of 150 mg/mL with inhibition diameter of 16.2, 15.6, and 15 mm, respectively (Table 1). Amoxicillin revealed potent inhibitory activity against *E. coli*, *P. aeruginosa* and *S. aureus* at the concentration of 150 mg/mL with inhibition diameter of 16.3, 16.4, and 18.5 mm, respectively. The findings investigated the tetracycline has more antibacterial activity than amoxicillin against Gram-negative bacteria, while amoxicillin showed more antibacterial activity than tetracycline against Gram-positive bacteria.

TABLE 1 Area of inhibitory zone of antibiotics against pathogenic bacteria

Bacterial species	Tetracycline (µg/mL)			Amoxicillin (µg/mL)		
	50	100	150	50	100	150
	Area of inhibition (mm)			Area of inhibition (mm)		
E. coli	12.4	14.1	16.2	12.1	14.0	16.3
P. aeruginosa	12.2	14.1	15.6	12.0	14.2	16.4
S. aureus	12.3	14.2	15.0	14.2	16.0	18.5

Sinameki (water extract) revealed weak inhibitory activity against all tested bacteria and on the other hand, *Sinameki* (methanol extract) showed potent inhibitory activity against *E. coli, P. aeruginosa,* and *S. aureus* with inhibition area of 8, 4, and 5 mm compared with the negative control (Table 2).

TABLE 2 Area of inhibitory zone of *Sinameki* against pathogenic bacteria

Plant Extract	Tested Bacteria	Inhibition Zone (mm)	Negative Control
Water	*S.aureus*	0	0
Water	*E.coli*	0	0
Water	*P. aeruginosa*	0	0
Methanol	*S. aureus*	8	0
Methanol	*E.coli*	4	0
Methanol	*P. aeruginosa*	5	0

Table 3 showed synergies between *Sinameki* extract and the used antibiotics. The combination of *Sinameki* extract with the antibiotics resulted in increasing inhibition of against all tested bacteria. The combination of *Sinameki* (water extract) with tetracycline showed significant inhibitory activity (10, 11.2, and 11 mm) against the whole used bacteria (*E. coli*, *P. aeruginosa,* and *S. aureus*) compared with both *Sinameki* (water extract) and tetracycline. Furthermore, the findings showed potent inhibitory activity of *Sinameki* (methanol extract) with tetracycline (13, 14, and 14 mm) against the tested bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*) compared with both *Sinameki* (water extract) and tetracycline. On the other hand, the combination of *Sinameki* (water extract) and amoxicillin resulted in increasing the activity against all tested bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*) with significant inhibitory activity (10, 13, and 12 mm) compared with *Sinameki* and amoxicillin. The combination of *Sinameki* (methanol extract) and amoxicillin showed potent inhibitory activity (13, 14, and 15 mm) against the tested bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*) compared with *Sinameki* and amoxicillin. Secondary metabolic compounds including anthraquiones, flavonoid, and coumarins can play an important role in increasing the inhibitory activity of *Sinameki* extract that particularly effect bacterial wall permeability,

and thus bacterial death in which interfering with DNA, adversely affecting the vital activities of the bacterial cell. The death of bacteria works also to destroy the wall of the bacteria by adhering to the wall proteins and affect the permeability, and therefore death of the cell. The death of bacteria works further to destroy the wall of the bacteria by adhering to the wall proteins and affect the permeability and therefore death of the cell.

TABLE 3 Area of inhibitory zone of extract and antibiotics against pathogenic bacteria

Plant Extract	Tested Bacteria	Plant extract Inhibition Zone (mm)	Negative Control	Tetracycline (10 µg) Inhibition Zone (mm)	Synergic effect of Tetracycline (10 µg) and Plant Extract (4 µg) Inhibition Zone (mm)	Amoxicillin (10 µg) Inhibition Zone (mm)	Synergic effect of Amoxicillin (10 µg) and Plant Extract (4 µg) Inhibition Zone (mm)
Water	*S.aureus*	0	0	6.2	10 ± 0.3	6.1	10 ± 0.2
Water	*E.Coli*	0	0	6.1	11.2 ± 0.5	6.0	13 ± 0.4
Water	*P. aeruginosa*	0	0	6.15	11 ± 0.7	8.2	12 ± 0.8
Methanol	*S. aureus*	5	0	6.2	13 ± 0.4	6.1	13 ± 0.1
Methanol	*E.coli*	8	0	6.1	14 ± 0.9	6.0	14 ± 0.3
Methanol	*P. aeruginosa*	4	0	6.15	14 ± 0.6	8.2	15 ± 0.5

Conclusion

In conclusion, we have investigated *in vitro* antibacterial activity of *Sinameki* extract against three strains including *E. coli*, *P. aeruginosa*, and *S. aureus. Sinameki* (water extract) revealed weak inhibitory activity against all tested bacteria, while the *Sinameki* (methanol extract) showed potent inhibitory activity against all tested bacteria. Our preliminary findings have indicated that *Sinameki* extract with tetracycline and amoxicillin can alter the cell membrane permeability of drug-resistant E. coli, *P. aeruginosa* and *S. aureus*. The combination of extract with tetracycline and amoxicillin leads to the significant inhibitory activity which achieving the dual therapeutic aims of a high antibacterial effect. Based on the findings, *Sinameki* extract can be used to treat infectious diseases caused by resistant microorganisms as antibacterial inhibitor.

Acknowledgements

Ahmed Mahal acknowledges the Chinese Academy of Sciences (CAS) for financial support through the CAS President's International Fellowship Initiative (2016PM032) and Cihan University-Erbil, as well.

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How to cite this article: Fouad Hussein Kamel*, Chnar Najmaddin Fathulla, Ahmed Mahal*, Anas Alkhouri, Shaho Ebrahim Rahman, Abdulkarim Sabr Osman. Antibacterial activity of *Cassia angustifolia.* Vahl (*Sinameki*) leaf extract against some pathogenic bacteria. *Eurasian Chemical Communications*, 2022, 4(11), 1108-1114.
Link: http://www.echemcom.com/article_152837.html