

Antibacterial Activity of Common *Varthemia*, *Varthemia iphionoides* Ethanol Extract Alone and in Combination with Cefotaxime

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Abstract: To assess the clinical utility of the crude ethanolic extract of the leaves of *Varthemia iphionoides* (Compositae), minimal inhibitory concentrations (MICs) were determined using agar dilution technique. Accordingly, The MICs of this plant extract ranged from 0.0313-1 mg/ml for three clinical *Staphylococcus aureus* strains and ATCC strains of *Bacillus subtilis* and *Staphylococcus epidermidis*, whereas *Escherichia coli* studied in this report was found to exhibit higher MIC value (4 mg/ml). The effect of combinations of ethanolic extract of *Varthemia iphionoides* and cefotaxime was investigated by means of fractional inhibitory concentration (FIC) indices. Using the FIC indices, synergistic interactions were observed against *B. subtilis* (ATCC 6633) and *S. aureus* strains (FIC indices of 0.75-0.875); while combinations against *E. coli* and *S. epidermidis* (ATCC 25923) exhibited antagonistic interactions (FIC indices of 2.5 to 16.4). Synergy was confirmed at cefotaxime concentrations corresponding to 1/2 MIC and an ethanol extract concentrations corresponding to 1/4 MIC and lower.

Key words: *Varthemia iphionoides* · Medicinal plants · Antibacterial activity · Herb-drug interactions · Plant extracts

INTRODUCTION

Interest in plants as sources of antimicrobial agents is growing. This is because plant-derived medicines have been part of traditional health care in most parts of the world and because the antimicrobial properties of plant-derived compounds are well documented [1-5]. In our continuing project to identify plant-derived compounds potentiated the antimicrobial effect of β -lactam antibiotics on bacteria, we screened an extract from dried leaves of *Varthemia iphionoides* Boiss and Bl.; *Varthemia iphionoides* Boiss and Bl. is widely distributed in Palestine and neighbouring countries. It is a perennial, bushy plant, 20-50 cm long, with a woody base and an aromatic, unbranching, hairy and sticky stems [6]. The aerial part of this plant is commonly used in the local folk-medicine for treatment of gastrointestinal disorders [7], the treatment of patients with diabetes mellitus [8] and healing eye inflammations [9]. The objectives of this study were: 1) to evaluate the *in vitro* antimicrobial activity of the ethanolic extract of the *Varthemia iphionoides* and 2) to investigate the synergic effects of this extract combined with cefotaxime against four different species of bacteria.

MATERIALS AND METHODS

Plant Material: Leaves of *Varthemia iphionoides* were collected from the northern area of Palestine in May 2009. Identification and classification of the plant material was performed at the Faculty of Science, An Najah N. University.

Plant Extract Preparation: The dried *Varthemia iphionoides* leaves were ground into fine powder and extracted with 80% ethanol. After filtration of total extracts, the extracts were evaporated to dryness at 40°C and weighed. Solutions of cefotaxime (Sigma-Aldrich Co. St., Louis, MO, USA) were obtained by dissolving in a Mueller-Hinton broth (Difco Laboratories).

Test Microorganisms: The following clinical strains were used in the study: *E. coli*, 3 strains of *S. aureus* (numbers 13, 15 and 16). Reference strains (*B. subtilis* ATCC 6633) and (*S. epidermidis* ATCC 25923) were also tested.

Determination of Minimal Inhibitory Concentrations: MICs were determined by the agar dilution method described by the National Committee for Clinical

Laboratory Standards [10]. Dilutions of the antibiotics, ranging from 0.064-0.0000625 mg/ml in Mueller-Hinton agar were prepared by incorporating the antibiotic stock solution into molten agar at 50°C. Dilutions of the extract ranging from 2-0.0313 mg/ml were also prepared by incorporation the extract in agar at 50°C. After pouring into plates and allowing the agar to set, the plates were inoculated with standardized inocula of the test bacteria of approximately 1×10^4 colony-forming units (CFU) per spot. Plates were incubated at 37°C for 24 h under aerobic conditions. MICs were the lowest concentrations of the antibiotic or extract resulting in complete inhibition of visible growth of the test organism.

Determination of Synergistic Activities

The Checkerboard Method: Synergistic effect between the plant extracts and cefotaxime was done using the agar dilution checkerboard method. This method utilized an inoculum of approximately 10^4 CFU/spot on Mueller-Hinton (MHA) plates mixed with the extract and the antibiotic in combination at concentrations ranging from $1/32 \times$ MIC to $2 \times$ MIC. The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotic and extract combination permitting no visible growth of the test organisms on the MHA plates after an incubation for 24 h at 37°C [11]. FIC indices were calculated using the formula: FIC index = (MIC of antibiotic in combination/MIC of antibiotic alone) + (MIC of extract in combination/MIC of extract alone). Combinations were classified as synergistic, if the FIC indices were < 1, additive if the FIC indices were = 1 indifferent if the FIC indices were between 1 and 2 and antagonistic if the FIC indices were >2 [12].

RESULTS

The MIC values of cefotaxime and ethanol extract of *Varthemia iphionoides* against the bacterial species used in this study are presented in Table 1. The extract showed different degrees of antibacterial activity in relation to the bacterial species used in this study. The MICs of

Table 1: Minimum inhibitory concentrations (MICs) of cefotaxime and *Varthemia iphionoides* ethanol extract

Test isolate	MIC values mg/ml	
	Cefotaxime	Ethanol extract
<i>Staphylococcus epidermidis</i> (ATCC 25923)	0.0005	0.0313
<i>Escherichia coli</i>	0.064	4
<i>Bacillus subtilis</i> (ATCC 6633)	0.032	1
<i>Staphylococcus aureus-13</i>	0.002	1
<i>Staphylococcus aureus-15</i>	0.002	1
<i>Staphylococcus aureus-16</i>	0.001	0.5

this plant extract ranged from 0.0313-1 mg/ml for three *S. aureus* strains and ATCC strains of *B. subtilis*, *S. epidermidis* (Gram-positive bacteria), whereas *E. coli* (Gram-negative bacteria) studied in this report was found to exhibit higher MIC value (4 mg/ml). The MICs of cefotaxime ranged from 0.0005-0.064 mg/ml for the tested bacterial species (Table 1).

The results of the checkerboard combinations are presented in Table 2. Synergistic interactions between cefotaxime and the extract of *Varthemia iphionoides* were observed against *B. subtilis* (ATCC 6633) and the *S. aureus* strains (FIC indices of 0.75-0.875), while combinations against *E. coli* and *S. epidermidis* (ATCC 25923) exhibited antagonistic interactions (FIC indices of 2.5 to 16.4). Synergy was confirmed at cefotaxime concentration corresponding to 1/2 MIC and an ethanol extract concentrations corresponding to 1/4 MIC and lower.

DISCUSSION

The species of *Varthemia iphionoides* is commonly utilized in Palestine in popular medicine, but its antibacterial activity has not been well studied. In a previous study, carried out to detect the antimicrobial activity of this plant [7], the crude water extract of the whole plant was found to show limited antibacterial activity. It has also been reported that the MICs of the ethyl acetate extract of this plant against pathogenic organisms ranged between 250 and 500 µg/ml [6].

Table 2: Fractional inhibitory concentration (FIC) values for the combinations between cefotaxime and the *Varthemia iphionoides* extract

Test isolate	Mean FIC (Antibiotic)	Mean FIC (Extract)	FIC Index	Interaction
<i>Staphylococcus epidermidis</i> (ATCC 25923)	0.5	15.9	16.4	Antagonism
<i>E. coli</i>	0.5	2	2.5	Antagonism
<i>Bacillus subtilis</i> (ATCC 6633)	0.165	0.689	0.854	Synergy
<i>Staphylococcus aureus-13</i>	0.002	1	0.75	Synergy
<i>Staphylococcus aureus-15</i>	0.002	1	0.75	Synergy
<i>Staphylococcus aureus-16</i>	0.001	0.5	0.875	Synergy

Our results indicate that *Varthemia iphionoides* extract acted best in relation to gram positive bacteria than gram negatives (Table 1). These results are consistent with previous reports on related plants regarding Gram-positive bacteria [13]. The resistance of *E. coli* to the tested extract was not unexpected as, in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [14].

Synergy between ethanolic extract of *Varthemia iphionoides* and cefotaxime using the FIC indices was observed in relation to *B. subtilis* (ATCC 6633) and *S. aureus* strains. This suggests the potential of this plant to improve the performance of cefotaxime. This fact was observed by others scientists, Sibanda and Okoh [4] showed potentials of synergy between acetone extracts of *Garcinia kola* seeds and amoxicillin, penicillin G as well as ciprofloxacin, tetracycline and chloramphenicol against pathogenic organisms.

The leaves of *Varthemia iphionoides* have been known to contain a number of antimicrobial compounds [6,7] such as flavonoids. The antimicrobial activity of flavonoids have been reported in other studies [15,16]. This would suggest that, the synergy with antibiotic observed in this study could be attributed to such compounds. These compounds have been shown to exert their antibacterial effect via cell membrane perturbations. Thus, coupled with the action of β -lactams on the transpeptidation of the cell wall could lead to an enhanced antimicrobial effect of the combination [17].

The synergy detected in this study was not observed in *E. coli*. This could be attributed to the permeability barrier provided by the cell wall of gram negative bacteria [14]. However, as *S. epidermidis* (ATCC 25923) exhibits low MIC for cefotaxime, synergism is less likely to occur. Usually, the combination of two agents exhibit significant synergism only if the test organism exhibits a high MIC to at least one of the agents [17].

Results of the present work showed that the ethanolic extract of *Varthemia iphionoides* potentiated the antimicrobial effect of cefotaxime. This probably suggests the therapeutic applicability of such compound in combination therapy.

REFERENCES

- Zuo, G.Y., G.C. Wang, Y.B. Zhao, G.L. Xu, X.Y. Hao, J. Hanand and Q. Zhao, 2008. Screening of Chinese medicinal plants for inhibition against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). *J. Ethnopharmacol.*, 120(2): 287-290.
- Chomnawang, M.T, S. Surassmo, K. Wongsariya and N. Bunyapraphatsara, 2009. Antibacterial activity of Thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*, 80(2): 102-104.
- Khan, R., B. Islam, M. Akram, S. Shakil, A. Ahmad, S.M. Ali, M. Siddiqui and A.U. Khan, 2009. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules*, 14: 586-597.
- Sibanda, T. and A.I. Okoh, 2008. *In vitro* evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics. *African J. Biotechnol.*, 7: 1672-1678.
- Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12: 564-582.
- Al-Dabbas, M.M., F. Hashinaga, S.A.M. Abdelgalei, T. Suganuma, K. Akiyama and H. Hayashi, 2005. Antibacterial activity of an eudesmane sesquiterpene isolated from common *Varthemia iphionoides*. *J. Ethnopharmacol.*, 97: 237-240.
- Afifi, F., S. Al-Khalil, B.K. Abdul-Haq, A. Mahasneh, D.M. Al-Eisawi, M. Sharaf, L.K. Wong and P.L. Schiff, 1991. Antifungal flavonoids from *Varthemia iphionoides*. *Phytotherapy Research*, 5: 173-175.
- Afifi, F., M. Saket, M. Jaghabir and D. Al-Eisawi, 1997. Effect of *Varthemia iphionoides* on blood glucose level of normal rats and rats with streptozocin-induced diabetes mellitus. *Current Therapeutic Research*, 58: 888-892.
- Abu-Rabia, A., 2005. Indigenous practices among Palestinians for healing eye diseases and inflammations. *Dynamis: Acta hispanica ad medicinae scientiarumque historiam illustrandam*, 25: 383-401.
- National Committee for Clinical Laboratory Standards, 1994. Performance for antimicrobial susceptibility testing. Standard M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Mandal, S., M.D. Mandal and N.K. Pal, 2004. Evaluation of combination effect of ciprofloxacin and cefazolin against *Salmonella enterica* serovar *typhi* isolates by *in vitro* methods. *Calicut Medical J.*, 2(2): e2.
- Kamatou, G.P.P., A.M. Viljoen, S.F. van Vuuren and R.L. van Zyl RL, 2006. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagnea* and *Leonotis leonurus*. *South African J. Botany*, 72: 634-636.

13. Bassam Abu-Shanab, B., G. Adwan, D. Abu-Safiya, N. Jarrar and K. Adwan, 2004. Antibacterial Activities of Some Plant Extracts Utilized in Popular Medicine in Palestine. *Turkish J. Biol.*, 28: 99-102.
14. Adwan, K. And N. Abu-Hasan, 1998. Gentamicin resistance in clinical strains of *Enterobacteriaceae* associated with reduced gentamicin uptake. *Folia Microbiol.*, 43: 438-40.
15. Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. *Intl. J. Antimicrobial. Agents*, 26(5): 343-356.
16. Sato, Y., H. Shibata, T. Arai, A. Yamamoto, Y. Okimura, N. Arakaki and T. Higuti, 2004. Variation in synergistic activity by flavone and its related compounds on the increased susceptibility of various strains of methicillin-resistant *Staphylococcus aureus* to β -lactam antibiotics. *Intl. J. Antimicrobial Agents*, 24(3): 226-233.
17. Esimone, C.O., I.R. Iroha, E.C. Ibezim, C.O. Okeh and E.M. Okpana, 2006. *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. *African J. Biotechnol.*, 5(11): 1082-1086.