

IDENTIFICATION OF A NOVEL SYNERGISTIC COMBINATION OF NATURAL EXTRACTS FOR INFECTION CONTROL IN DIABETIC ULCERS



A ZALUVIDA COMPANY.

WHATMOUGH, A., WILLIAMS, D. M., EVANS, G. AND GRAZ, M.

NEEM BIOTECH, WALES, UK.

BACKGROUND

By 2030, 550 million people will suffer with diabetes, 25% of which will develop diabetic leg and foot ulcers that are characterised by persistent, polymicrobial infections and antibiotic resistance. Within these, up to 80% of challenging wounds may have a bacterial infection.

We previously demonstrated that, *in vitro*, two natural extracts from garlic and sandalwood oil inhibit the growth of five isolates of prolific, wound associated, often multidrug resistant bacteria: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterococcus faecalis*.

AIMS

The aims of this study were two-fold; (i) To ascertain the presence of the synergistic effect between a defined garlic extract (GE) and three commercially available sandalwood oils (SS1, SS2 and SS3), and (ii) to identify the combination with the greatest antimicrobial effect against chronic wound-associated bacteria.

METHODS

A microdilution checkerboard method¹ assessed the antimicrobial efficacy of the natural extract combinations. Inhibition of bacterial growth was assessed by monitoring optical density in comparison to an untreated control. Bacterial isolates used were *P. aeruginosa* NCTC 10662, *S. aureus* NCTC 8532, MRSA ATCC 33591, *E. faecalis* NCTC 12697 and *A. baumannii* NCTC 12156.

The synergistic relationship of the combinations were assessed by applying the Fractional Inhibitory Concentration Index (FICI)¹ to the microdilution checkerboard data. A synergistic relationship is indicated by an FICI value of ≤ 0.5 . Heat mapping was performed by averaging the results of the checkerboard microdilution assay and applying a range of colours set within the highest and lowest growth inhibition values.

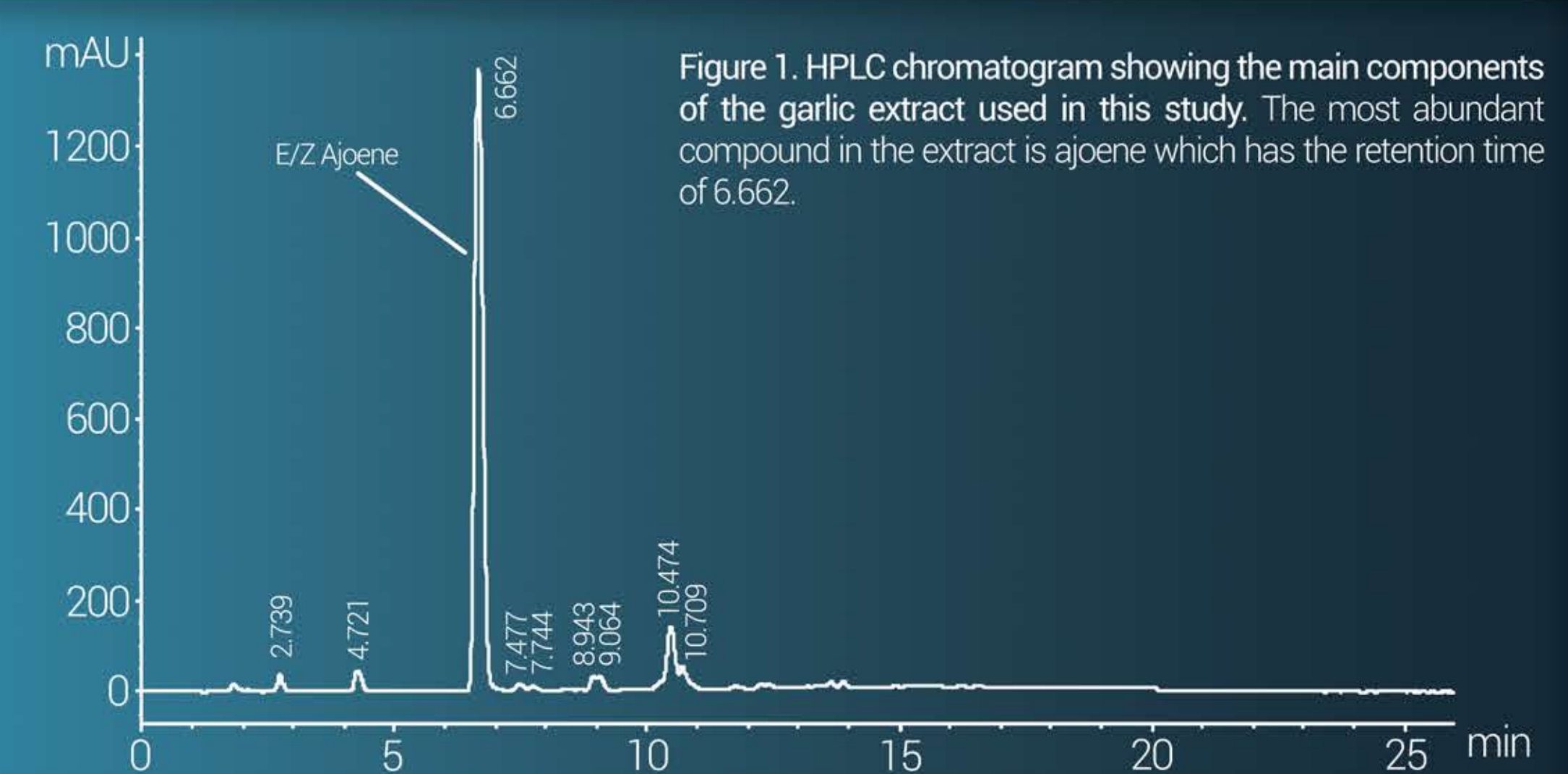


Figure 1. HPLC chromatogram showing the main components of the garlic extract used in this study. The most abundant compound in the extract is ajoene which has the retention time of 6.662.

RESULTS

SS1 and GE had the lowest FICI values exhibiting the highest level of synergy against *S. aureus*, MRSA, *E. faecalis*, but had no effect on *P. aeruginosa* or *A. baumannii*.

SS3 and GE also exhibited synergy against four of the five isolates, with no effect on *A. baumannii*.

An FICI value for SS2 could not be calculated as no MIC was obtained within the ranges tested.

Table 1. The FICI values for two of the sandalwood samples in combination with garlic extract. FICI values of ≤ 0.5 indicates a synergistic relationship. *ND (Not Determined); the sandalwood samples did not reach an MIC50.

Bacteria	FICI Value	
	Sandalwood Sample 1 and Garlic Extract	Sandalwood Sample 3 and Garlic Extract
<i>P. aeruginosa</i>	ND*	0.141
<i>S. aureus</i>	0.094	0.375
MRSA	0.156	0.250
<i>E. faecalis</i>	0.078	0.188
<i>A. baumannii</i>	ND*	ND*

BACTERIAL INHIBITION

2a.		Sandalwood Sample 1 ($\mu\text{g/mL}$)							
		0	7.8125	15.63	31.25	62.5	125	250	500
Garlic Extract ($\mu\text{g/mL}$)	0	-1%	3%	1%	-4%	-4%	0%	53%	62%
	7.8125	10%	12%	21%	16%	22%	32%	67%	73%
	15.63	9%	17%	27%	21%	27%	41%	69%	73%
	31.25	21%	26%	22%	27%	33%	57%	72%	73%
	62.5	40%	54%	53%	51%	54%	63%	76%	73%
	125	74%	77%	77%	75%	79%	79%	79%	85%
	250	76%	83%	78%	80%	82%	82%	81%	82%
	500	81%	88%	89%	82%	91%	90%	94%	96%

Table 2. Heat maps of the percentage inhibition compared to the untreated control averaged across all five bacterial isolates. Colours are relative and comparable with the most intense green being the highest percentage inhibition of growth and the most intense red being the lowest percentage inhibition of growth. 2a. Sandalwood sample 1 and garlic extract. 2b. Sandalwood sample 3 and garlic extract.

2b.		Sandalwood Sample 3 ($\mu\text{g/mL}$)							
		0	7.8125	15.63	31.25	62.5	125	250	500
Garlic Extract ($\mu\text{g/mL}$)	0	-4%	1%	0%	18%	68%	75%	77%	75%
	7.8125	8%	13%	27%	43%	70%	75%	78%	74%
	15.63	15%	29%	32%	58%	72%	75%	75%	76%
	31.25	35%	32%	37%	65%	73%	78%	81%	77%
	62.5	52%	61%	60%	73%	77%	83%	88%	85%
	125	80%	81%	81%	85%	80%	92%	99%	103%
	250	85%	84%	90%	88%	93%	93%	95%	106%
	500	93%	99%	95%	106%	102%	102%	98%	100%

CONCLUSIONS

A synergistic relationship was demonstrated between GE and both SS1 and SS3, with SS3 slightly superior to SS1. This demonstrates enhanced antimicrobial properties against four of five bacterial isolates prevalent in challenging wounds.

The combination of GE and SS1/3 could be exploited in a topically applied product for the treatment of challenging wound infections. The combination was effective against a range of bacteria known to be present in challenging wounds. Further work is ongoing to understand its full potential as a novel therapeutic approach in this area of high unmet medical need.