Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.22, 2016

Correlation Between Vicia ervilia L. Willd. Antibacterial Activity and Its Phenolic Content

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Abstract
Context: Plants are important in devising new antibacterial drugs. Unlike several fabaceae seeds, *Vicia ervilia* L. Willd., is not used up till now for human consumption. **Objective:** evaluate antibacterial potential of the seeds and correlate it, if any, with its phenolics. **Methods:** Seeds ethanol (SEE), aqueous (SAE), and methanol (SME) extracts were prepared. Their total phenolic content (TPC) was determined spectrophotometrically. Antibacterial activity against ten pathogenic bacteria (*Mycobacterium africanum*, *M. bovis*, *M. caprae*, *M. microti*, *M. orygis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhimurium*) was evaluated using agar well diffusion assay. Also the minimum inhibitory and bactericidal concentrations (MIC & MBC) were also determined. **Results:** Total phenolic content of SME is significantly ($p<0.001$) higher than that of SEE (4.8 and 2.5µg/mg gallic acid equivalent respectively). SME and SEE significantly inhibit the growth of all tested strains except *M. caprae* and *P. aeruginosa*. *V. ervilia* L. **Conclusion:** The seeds significant antibacterial activity was attributed to its phenolics.
Keywords: phenolics, antibacterial activity

1. Introduction

Vicia ervilia L. Willd. (*Ervium ervilia* L., kursene كرسنه), Fabaceae, is an annual herb distributed in the Mediterranean coastal region. The seeds are used in several countries as stock feed (Garlinge & Perry 1993; Enneking & Francis 1997; Haddad 2006; Sadeghi et al. 2009). However, to the best of the authors' knowledge, only one report concerning the medicinal importance of *V. ervilia* for human beings was traced (Fornstedt & Porath 1975). No reports were traced neither on the antimicrobial potential or quantitative aspects of phenolics in *V. ervilia* seed. This stimulated the authors to estimate its total phenolic content (TPC) and evaluate its antimicrobial activity.

2.Experimental

2.1Plant material

Samples of *V. ervilia* seeds were imported from Jordan in July 2013. They were cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza. Photos of the cultivated plant were sent to Kew Garden, England to confirm their identity. Identification was studied by same authors in a previous publication (Okba et al. 2014). Voucher samples were deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University (herbarium no. 14.4.2013.2).

2.2 Preparation of extracts

Seeds were powdered and submitted to three different methods of extraction. First portion was extracted with ethanol (70%) by percolation at room temperature. The solvent was evaporated at 45°C under vacuum using rotary evaporator to yield seeds ethanol (70%) extract (SEE). The seed aqueous extract (SAE) was prepared by extracting the powdered seeds with water under reflux for 4 hr. The seed methanol extract (SME) was prepared by continuous extraction in Soxhlet for 10 hours. The mixture in each case was filtered. The filtrate was evaporated at 45°C under vacuum using rotary evaporator.

2.3 Determination of total phenolic content (TPC)

Spectrophotometric determination of TPC was carried out according to the European pharmacopoeia (Druckerei 2002). Total phenolics were expressed as µg of gallic acid (Sigma-Aldrich, Taufkirchen, Germany) equivalents/mg of dry extract.

2.4 Antibacterial activity: was determined using the agar well diffusion method as described by Holder & Boyce 1994. Ten pathogenic bacteria were used *Mycobacterium africanum*, *M. bovis*, *M. caprae*, *M. microti*, *M. orygis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium* (RCMB 0023645, 0023642, 00236940, 00236948, 00236941, 000106, 010010, 010043, 010052, 010072 respectively). The tested bacteria were subcultured on nutrient agar medium (Oxoid laboratories, UK). Gentamicin was used as reference standard. Antibacterial activity was determined by measuring zone of

inhibition (Agwa *et al*. 2000). The minimum inhibitory concentration (MIC) was estimated according to (Doughari 2006).

3. Results and discussion

TPC of different extracts of the seeds was estimated (Table 1). SME showed a significant higher ($p<0.001$) TPC than SEE and SAE (4.8, 2.5 and 2.7 µg/mg gallic acid equivalent respectively). Thus, methanol is preferred for extraction of *V. ervilia* seeds phenolics.

Table 1. TPC of different *V. ervilia* seed extracts

Extract	Absorbance*	S.E.	TPC (µg/mg) Gallic equivalent
Seed:			
SME	0.097	0.000245	4.80
SEE	0.051	0.000447	2.50
SAE	0.056	0.000374	2.75

*average of five determinations; S.E., Standard error; TPC, total phenolic content. SME: seed methanol extract; SEE: seed ethanol extract; SAE: seed aqueous extract.

Antibacterial activity of *V. ervilia* seeds were examined (Table 2). All the tested extracts, except SAE, were active against *M. africanum*, *M. bovis*, *M. microti*, *M. orygis*, *S. aureus*, *S. pneumonia*, *E. coli* and *S. typhimurium*. Tested extracts were inactive against *M. caprae* and *P. aeruginosa*. It did mean that they were resistant to *V. ervilia* extracts or that they necessitated higher concentrations.

SME was more active than the SEE against all tested bacteria this is attributed to its higher TPC. It is well established that phenolic compounds possess antimicrobial activity (Lucchini et al. 1990; Alberto et al. 2006). This is matched with findings of Akroum *et al*. 2009 who found that methanol extract of *V. faba* had higher antimicrobial activity than its ethanol extract.

SME activity against *S.p*., *M.m., M.a.* and *E.c*. reached 95, 88,88, and 83 % of R.S.G., respectively. *S. pneumoniae* is a major cause of pneumonia in the late 19th century (Abut 2008). *E. coli* are among bacteria that cause gastroenteritis, urinary tract infections and diarrhea (Nataro & Kaper 1998; Jarraud et al. 2001; Becker et al. 2003; Todar 2007). *M. africanum* is a species of *Mycobacterium* that is most commonly found in West African countries causing up to half of human tuberculosis (TB) in West Africa (De Jong *et al.* 2009). Further studies are therefore required to detect potency of *V. ervilia* extracts in treating infections caused by the previously mentioned bacteria.

It worth to note that *M. bovis* was the most sensitive tested *Mycobacterium* to *V. ervilia* samples. SME inhibited its zone of growth by 116% of R.S.G. zone of inhibition with MIC (0.98 µg/ml), a value which is lower than that of R.S.G. (1.95 µg/ml). *M. bovis* is the causative agent of tuberculosis in cattle (known as bovine TB) (Grange 1996). The pronounced activity of the SME against *M. bovis* may suggest its incorporation in the preparations used in treatment of cattle with TB infection. *M. bovis* can also jump the species barrier and cause tuberculosis in humans (Grange 1996). So further studies for the use of *V. ervilia* extracts in treatment of human tuberculosis is recommended after testing its activity against *M. tuberculosis* especially that some of *V. ervilia* extracts were active against *M. caprae* which can cause tuberculosis (Rodríguez et al. 2009). Studying MIC and MBC of the tested extracts revealed that SME excreted bactericidal activity while, SEE is bacteriostatic.

SAE is inactive against all tested strains. It is well documented that *V.ervilia* seeds have to be soaked in boiling water before being used as stock feed (Berger et al. 2003; Sadeghi et al. 2004) to remove the antinutritional factor, canavinine. The lack of antibacterial activity of SAE confirm that the seed antibacterial phytochemicals will not be lost after the detoxification process.

This is the first report on antibacterial activity of such promising seeds, *V. ervilia.*

Table 2. Antibacterial activity of *V. ervilia* seed extracts

		Diameter of inhibition zone (mm) %*					MIC and MBC (µg / ml)			
		Seeds			R.S.G.		Seeds			R.S.G
		SME	SAE	SEE			SME	SAE	SEE	
Gram –positive	*M. a.*	21	-	14	24	MIC	0.98	-	125	0.06
		88%		58%	100%	MBC	0.98	-	250	0.06
	M. b.	22	-	12	19	MIC	0.98	-	125	1.95
		116%		63%	100%	MBC	0.98	-	250	1.95
	M. m.	21	-	14	24	MIC	0.49	-	125	0.06
		88%		58%	100%	MBC	0.49	-	250	0.06
	M. o.	21	-	13	25	MIC	0.49	-	125	0.015
		84%		52%	100%	MBC	0.49	-	250	0.015
	S. a.	17	-	12	25	MIC	15.63	-	125	0.03
		68%		48%	100%	MBC	15.63	-	250	0.03
	S. p.	19	-	13	20	MIC	3.9	-	125	1.95
		95%		65%	100%	MBC	3.9	-	250	1.95
Gram –negative	*E. c.*	20	-	12	24	MIC	7.81	-	125	0.06
		83%		50%	100%	MBC	7.81	-	250	0.06
	S. t.	21	-	13	26	MIC	0.98	-	125	0.03
		81%		50%	100%	MBC	0.98	-	250	0.03

* Mean of inhibition zone in mm beyond well diameter (6 mm), concentration of tested samples: (1mg/ml). SEE: seed ethanol extract.; SME: seed methanol extract; SAE: seed aqueous extract. *M. a.*, *Mycobacterium africanum*, *M. b.*, *Mycobacterium bovis*, *M.c.*, *Mycobacterium caprae*, *M.m., Mycobacterium microti*, *S.a.*, *Staphylococcus aureus*, *S.p., Streptococcus pneumoniae*, *E.c., Escherichia coli*, *S.t., Salmonella typhimurium*. R.S.G., reference standard gentamycin.

4. Conclusion

It was found that *V. ervillia* L. seeds possess a wide spectrum of antibacterial activity. The plant newly discovered significant antibacterial activity is attributed to its phenolic content. Methanol is more preferred than water and ethanol (70%) in extraction of the seeds phenolics. The plant can be used to relive different illness after extensive clinical studies. The only restriction for the seeds use, its canavinine content, can be easily removed by soaking in boiling water before use without loss of its newly explored antibacterial potential.

Acknowledgments

The Authors would like to thank Dr. Shahira El-Komy Associate Prof. Pharmacognosy Department, Faculty of Pharmacy, Cairo University for her help in revising the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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