Phenolic contents and antimicrobial activity of squirting cucumber *(Ecballium elaterium)* extracts against food-borne pathogens

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Abstract: Squirting cucumber (*Ecballium elaterium*), is an indigenous species of the south of Europe and the Mediterranean basin, sometimes cultivated for its use as a medicinal plant. This study compared phenolic contents in *Ecballium elaterium* organs and their antimicrobial activities against some foodborne pathogens. Our results indicated that the plant organs had different total polyphenol contents (ranging from 6.744 to 46.848 mg GAE g^{-1} DW) the leaves and fruits contained about 6-fold higher phenol contents than the root. The same tendency was observed for flavonoid and tannin levels. An interesting antimicrobial activity was also observed against the food pathogens at concentrations ranging from 0.004 to 2.5 mg ml^{-1}. *Ecballium elaterium* extracts might therefore be a potential source of preservative candidates for use in food or pharmaceutical industries.

Keywords: squirting cucumber; *Ecballium elaterium*; phenolic composition; antimicrobial activity; food pathogens.

INTRODUCTION

Food spoilage and food-borne pathogens development on food products has a direct effect on the decrease of the nutritional quality by consuming protein, fat and carbohydrate present in these products which subsequently causes food discolor ration, biochemical changes and toxicity, in addition of their adverse economic consequences. Many bacterial and fungal contaminants are able to produce some highly toxic secondary metabolites, like mycotoxins, that are capable of causing disease and death in humans (You, 2006).

Actually, many preservation methods were employed in food industry including low-temperature storage, vacuum package, irradiation… but the use of chemical preservatives remains the most employed method in agro-industry. However, the safety problems related to the use of chemical preservatives are receiving growing attention. Therefore, many research teams have focused on the development of safety preservation procedures employing naturally derived substances such as salt, sugars, vinegar and natural extracts from dietary plants. The richness of these dietary plants on phenolic compounds such as tannins and flavonoids, known for their several biological effects including antimicrobial properties, can have a direct impact on reducing the health hazards and economic losses due to food-borne pathogens.

Ecballium elaterium (L.) also known as "wild or squirting cucumber" is a plant from the cucumber family (*Cucurbitaceae*), this family contains a large number of edible crops such as the cucumbers, pumpkins and melons. The fruit of the plant squirts violently a jet of liquid along with the seeds on ripening, by detonating the high pressure developed within the fruit, and is therefore also known popularly as exploding or squirting cucumber.

Literature provided abundant information about health benefits of *E. elaterium*. The plant roots were employed for its analgesic properties and to treat hemorrhoids in Turkish folk medicine. While the fresh fruit juice was commonly used to treat jaundice, nocturia, lumbago, otalgia (Toker *et al.,* 2003), sinusitis and painful joints by nasal aspiration. However, it was associated with undesirable effects such as the irritation of nasal mucous membranes, uvular angioedema, drooling, dysphagia and vomiting (Raikhlin-Eisenkraft and Bentur, 2000). *E. elaterium* fresh juice has several toxic and beneficial effects including analgesic, antipyretic, and anti-inflammatory effects (Agil *et al.,* 1995). An antipro liferative activity against some types of cancer cells was also attributed to cucurbitacins and their glycosylated derivatives. But his most interesting potential activity can be antiviral (El-Haci and Bekkara, 2011).

The aims of our study were to evaluate the antibacterial activity of *E. elaterium* part extracts against general food-borne pathogens and to determine total polyphenol, flavonoid, and tannin contents at organ level (leaves, flowers, roots and fruits).

MATERIALS AND METHODS

Plant collection and extraction

The plant *Ecballium elaterium* (Cucurbitaceae), authenticated by Professor Abderazzak Semaoui from The Biotechnologic Center Borj-Cedria Technopark was collected from Béja in the Northwest of Tunisia in February 2009. A voucher specimen (E.E.C01-01) was deposited at the herbarium in the Higher Institute of

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Biotechnology of Béja, Tunisia. The different organs of the plant were extracted by overnight maceration in solvents with different polarities at a rate of 10 g of dried powder in 30 ml of organic solvent. Each extract was then filtered, concentrated under vacuum and finally dried to remove solvent traces. The extracts obtained were then used for the further experiments.

Polyphenol extraction

The air-dried leaf, root, fruits and (1 g of each ground organ) were separately extracted by stirring with 10 ml of methanol-water (8:2, v/v) for 30 min. The extracts were then filtered, evaporated under vacuum to dryness and stored at 4 °C until analyzed.

Total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteu reagent, as described by Mhamdi *et al*, (2010). Total phenolic contents of each organ (three replicates per treatment) were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW) through the calibration curve with gallic acid. The calibration curve range was 50-400 mg/ml ($r^2 = 0.99$). All samples were performed in triplicates.

Total tannin contents

Total tannin contents were determined by the Folin–Ciocalteu procedure, as described by Mhamdi *et al*, (2010). Calculated values were subtracted from total polyphenol contents and the amount of total tannins expressed as mg gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW) using a calibration curve with gallic acid ranging from 50 to 400 mg ml^{-1} ($r^2 = 0.99$).

Total flavonoid contents

The colorimetric assay developed by Dewanto *et al.* (2002) was used to evaluate total flavonoid contents of *E. elaterium* organs. 250 µl of the methanolic extract appropriately diluted, were mixed with 75 µl $NaNO_2$ (5%). After 6 min, 150 µl of 10% $AlCl_3$ and 500 µl of NaOH (1 M) the 2.5 ml of distilled water were respectively added to the mixture. The mixture absorbance was then read at 510 nm. Total flavonoid contents of each organ (three replicates per treatment) were expressed as mg catechin equivalents per gram of dry weight (mg CE g^{-1} DW) through the calibration curve with catechin. The calibration curve range was 25-500 mg ml^{-1}.

Microbial strains

Representative food borne bacteria (*Listeria monocytogenes, Salmonella typhimurium Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) were used to evaluate the antimicrobial properties of *E. elaterium* organs. The yeast strain *Candida albicans* ATCC 10231 was also used to estimate the antifungal activity of squirting cucumber organs.

Antimicrobial assays

Exponential bacterial cultures were cultured as described previously (Abbassi *et al*, 2008). Briefly, 50 µl of 2-fold serial dilutions of each extract were mixed with 50 µl of microorganism suspension (10^6 cfu/mL) in 96-well microtitration plates. The plates were incubated overnight and the absorbance at 630 was measured using a microplate spectrophotometer. MICs were expressed as the lowest concentration of extract that inhibited microbial growth completely and as the average value from three independent experiments. Formaldehyde (0.7%) and H_2O were used as positive and negative controls respectively. Because of the higher color intensity of leaf extracts which interfered with optical density, the antimicrobial activity of these extracts were not being presented.

Hemolysis assay

The hemolytic activity of *Ecballium elaterium* methanolic extracts was evaluated using human erythrocytes. Different extracts at the concentrations ranging from 0.05 to 10 mg ml^{-1}, were incubated with washed erythrocytes (10^8 cells) in PBS (Dulbecco's phosphate-buffered saline) pH 7.4 (100 µl) for 1 h at 37 °C. After centrifugation (1000 g for 5 min), the absorbance at 450 nm of the supernatant was measured. A parallel erythrocytes incubation in the presence of Triton X 0.1% and PBS served as controls inducing 100% and 0% hemolysis, respectively. Extracts hemolytic activities were expressed as LC_{50} corresponding to the concentration inducing 50% hemolysis.

RESULTS

Total polyphenol, flavonoid and tannin contents of E. elaterium organs

The total phenol contents of the different organs of *E. elaterium* were shown in table1. Total phenols (r^2 = 0.9956) were expressed in terms of gallic acid (GA) equivalents per gram of the dry weight of the organ used (mg GAE g^{-1} DW). The mean values of phenols ranged from 6.744 to 46.848 mg GAE g^{-1} DW. The plant organs had different total phenol contents, the leaf and fruit containing about 6-fold higher phenol contents than the root and about 4-fold higher phenol contents. These results are in agreement with recent study of El-Haci and Bekkara (2011) reporting the antioxidant properties of *Ecballium elaterium* stems and leaves.

Accordingly, leaves exhibited the highest total flavonoid contents (17.38 mg CE g^{-1} DW), followed by the fruits (9.918 mg CE g^{-1} DW) and the lowest level was found in flowers (2.526 mg CE g^{-1} DW). Tannin contents were present in all studied squirting cucumber organs, but in lower abundance than flavonoids, except in flowers and

roots (table 1). We have observed that fruit and leaves showed the highest tannin content (4.29 and 3.54 mg GAE g^{-1} DW respectively).

Antibacterial and hemolytic activities

Ecballium elaterium part extracts tested showed various degrees of inhibition against the 6 bacterial strains tested using the microdilution method as presented in table2. We have observed that the type of solvent used to extract the different parts of *Ecballium elaterium* can have a great impact on the antimicrobial activity. This difference could be attributed to the selectivity of extraction. In fact the extraction of *E elaterium* with polar solvents (methanol ethanol, acetone) resulted in products with greater overall antimicrobial activity as compared with extraction with non polar ones (chloroform and ether/ petrol) (Thongson *et al.,* 2004).

Various plant organs (fruits, seeds and flowers) of *E. elaterium* showed different levels of antibacterial activity against tested strains used. In fact *E. elaterium* flower extracts exhibited a good antibacterial activity essentially against food borne pathogens *Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogenes* at low MICs ranging from 0.31 to 0.04 mg ml^{-1} (table 2). Root extracts exhibited also good antibacterial activity against all Gram positive bacteria tested at concentrations ranging from 2.5 to 0.004 mg ml^{-1}. Our results indicated also that

Table 1: Total phenolic, tannin and flavonoid contents of *Ecballium elaterium* organs

Organs	Polyphenol contents (mg GAE g^{-1} DW)	Flavonoid contents (mg CE g^{-1} DW)	Tannin content (mg GAE g^{-1} DW)
Fruit	43.61 ± 1.65	9.91 ± 0.51	4.29 ± 0.33
Leaves	46.84 ± 2.50	17.38 ± 1.65	3.54 ± 0.66
Flowers	11.56 ± 0.51	2.52 ± 0.33	3.09 ± 0.66
Roots	6.74 ± 0.51	4.42± 0.51	2.09 ± 0.33

Total phenolic and tannin contents were expressed by milligram gallic acid equivalent per gram dry weight (mg GAE g^{-1} DW); total flavonoids were expressed by milligram catechin equivalent per gram dry weight (mg CE g^{-1} DW). Data are given as mean of three replicates ± S.E.M.

Table 2: Antibacterial activity of *Ecballium elaterium* organ extracts

Solvent extract	Plant part extracted	MIC (mg/ml)*					
		E.c	*P.a*	*S. t*	*L.m*	*E. f*	*S. a*
Methanol	Root	ND	ND	1.25±0.16	0.15±0.00	0.078±0.00	2.5±0.33
	Fruit	ND	ND	ND	0.15±0.00	2.5±0.66	0.62±0.00
	Flowers	ND	ND	0.078±0.00	0.06±0.00	ND	0.15±0.02
Ethanol	Root	ND	ND	1.25±0.33	0.31±0.08	ND	0.31±0.08
	Fruit	ND	ND	2.5±0.33	ND	ND	0.62±0.08
	Flowers	ND	ND	0.04±0.00	0.04±0.00	ND	0.04±0.00
Acetone	Root	ND	ND	0.31±0.08	0.15±0.02	0.06±0.00	ND
	Fruit	ND	ND	ND	0.31±0.00	ND	1.25±0.33
	Flowers	ND	ND	0.15±0.08	ND	ND	0.15±0.02
H_2O	Root	ND	ND	1.25±0.33	0.06±0.00	0.004±0.00	0.62±0.00
	Fruit	ND	ND	ND	ND	ND	ND
	Flowers	ND	ND	ND	ND	ND	ND
Ethyl acetate	Root	ND	ND	ND	ND	ND	ND
	Fruit	ND	ND	0.15±0.08	0.15±0.00	2.5±0.33	0.15±0.00
	Flowers	ND	ND	0.15±0.08	0.06±0.00	ND	0.15±0.00
Chloroform	Root	ND	ND	1.25±0.00	ND	ND	0.15±0.00
	Fruit	ND	ND	ND	ND	2.5±0.66	ND
	Flowers	ND	ND	0.15±0.08	0.15±0.02	ND	0.31±0.00
Ether /petrol	Root	ND	ND	ND	ND	0.06±0.02	ND
	Fruit	ND	ND	ND	ND	ND	ND
	Flowers	ND	ND	ND	ND	ND	0.15±0.00

Values represent the means from three independent experiments performed in triplicate.
ND : Not detected; *P. a* : *Pseudomonas aeruginosa* ATCC 27853; *E.c* : *Escherichia coli* ATCC 25922, *S.t : Salmonella typhimurium, S.a : Staphylococcus aureus* ATCC 25923; *L.m*: *Listeria monocytogenes* and *E.f* : *Enterococcus faecalis* ATCC 29212.

the root methanolic extract exhibited antifungal activity against *Candida albicans* ATCC 10231 at a MIC of 2.5 mg ml^{-1}, these results are in agreement with recent works of Adwan and collaborators (2011), indicating that the methanolic extract of *E. elaterium* exhibited anticandidal activity (Adwan *et al.,* 2011). Contrary to literature reports dealing about *E. elaterium* toxicity, we have observed that *E. elaterium* part extracts don't exhibited hemolytic activity against human erythrocytes at concentrations ranging from 10 to 0.004 mg ml^{-1} except for root aqueous extract (LC_{50} = 0.5 mg ml^{-1}) (data not shown).

DISCUSSION

Long known for its anti-inflammatory, analgesic and carcinogenic properties, we have report for the first time in this study, that squirting cucumber exhibited also interesting activity against some of the representative food-borne pathogenic bacteria such as *S. aureus*, *L. monocytogenes*, *S. typhimurium*. We have also observed that among the six bacteria tested, in the present study, Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*) were the most sensitive to *E. elaterium* extracts, while *E. coli* and *Pseudomonas aeruginosa* were the most resistant. The lowest sensitivity of Gram negative bacteria tested in this study to the extract may be due to their cell wall structure and outer membrane (Zaika, 1988). It has been reported that Gram-negative bacteria are generally less sensitive to herb extracts than Gram-positive bacteria, due to the significant outer layers differences between Gram+ and Gram- bacteria. These last ones possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria (Duffy and Power, 2001; Shan *et al.,* 2007). The Gram- bacterial resistance against antibacterial agents is related to the hydrophilic character of their outer membrane which is rich in lipopolysaccharide molecules, that serves as a barrier to the penetration of these antibacterial agents.

The antibacterial properties of *E. elaterium* extracts can be due to the richness of its organs on polyphenolic compounds. Many studies have demonstrated that good linear relationships exist between antibacterial activity and the high level of phenolic components and emphasized the importance of several classes of polyphenol such as phenolic acids, flavonoids and tannins phenolic compounds in plant defense mechanism against pathogenic microorganisms, insects, and herbivores. However, we have observed that flower extracts exhibiting the highest antibacterial activity, as compared with the other tested organs, have lower amounts of polyphenolic contents. These findings can be explained by the nature of the components implicated in the antimicrobial activity. We cannot exclude that components in lower amounts also contributed to the antimicrobial activity of plant extracts (Melliou *et al.,* 2007). It has been also observed that the minor components might be involved in some type of synergism with the other active compounds (Marino *et al.,* 2001).

The richness of aerial organs in phenolic classes as compared to roots may be attributed to the irradiation with light that affects the phenolic content of the organs. There is a well-established positive relationship between natural radiation and the synthesis of phenolics produced by plants (Niknam and Ebrahimzadeh, 2002). These differences in pholyphenol distribution between plant organs were the result of organ adaptation to the environment conditions by the contribution of phenolics in the protection of plant aerial organs against photooxidation. An adaptive interpretation of this differential organ response has been attributed to the involvement of phenolics in the protection of exposed tissues against photooxidation (Niknam and Ebrahim zadeh, 2002). Garcia-Macias *et al.,* 2007 have demon strated that the increased concentrations of total phenols and the main flavonoids for many plant species was most likely due to the leaves exposure to increased levels of UV. Thus confirming that leaf function serves as defense mechanism against UV damage. These secondary metabolites and related phenolic compounds are most likely the major source of UV-B absorption in leaf epidermis.

The antimicrobial properties of polyphenols occurring in medicinal plants and vegetable foods have been widely investigated against a broad range of microorganisms. Many studies have demonstrated that plants of *Cucurbitaceae* family were found to contain a number of naturally related triterpenes, known collectively as cucurbitacins. These compounds are responsible for the bitterness in vegetables like cucumber, squash, eggplants, melon, pumpkin and gourds. Cucurbitacins are thought to protect the plant from herbivores and are toxic to many animals (but the taste is so bitter, it's rare for poisoning to occur) (Sharma *et al.,* 2006). This secondary metabolite family was also known for its antibacterial activity (Sharma *et al.,* 2006). We can then conclude that in addition of their richness on polyphenolic contents, the cucurbitacin secondary metabolites can also been implicated on the antibacterial activity observed of wild cucumber extracts studied in this investigation against food pathogens tested. Certain plant secondary metabolites can therefore be used as potential candidates for the development of natural and safe food preservatives, or to develop innovative alternatives for the treatment of multidrug resistant bacteria.

CONCLUSION

The use of biopreservatives has gained increasing attention in recent years, as they enhanced safety of foods

and are defined to extend its shelf life by the use of natural products. *Ecballium elaterium* extracts demonstrated inhibitory activity against some food pathogens, which support the possible use of wild cucumber extracts for food preservation, where microbial contaminants cause severe loss of food quality, edibility and nutritional value.

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