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Original Research Article

Chemical composition and biological activities of n-butanol extract of *Lepidium sativum* L (Brassicaceae) seed

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Abstract

Purpose: *To investigate the chemical composition, antioxidant properties and antibacterial effects of n-butanol extract of Lepidium sativum (Brassicaceae) seed.*
Methods: *The antioxidant activity of extracts of the seed obtained by maceration was determined by 1,1-diphenyl-2 picrylhydrazyl (DPPH), reducing power, and β-caroten/linoleic acid assays. The antimicrobial activity of the extract was tested against five strains of pathogenic microorganisms, viz, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300, Enterococcus feacalis ATCC 29212, Escherichia coli ATCC 25992 and Pseudomonas aeruginosa ATCC 27852. Its phytochemical composition was determined by liquid chromatography-mass spectrometry (LC-MS).*
Results: *A total of 17 compounds were identified by LC-MS, among which four compounds were kaempferol-hexose-rhamnose and three derivatives of kaempferol-rhamnose-hexose (benzoyl hexose) were previously unknown. The extract had better antioxidant than antibacterial properties.*
Conclusion: *These results indicate that n-butanol extract of Lepidium sativum seeds possesses good antioxidant properties which are most likely due to the presence of quercetin and kaempferol. Thus, these compounds account for the use of Lepidium sativum in traditional medicine.*

Keywords: *Lepidium sativum, Quercetin, Kaempferol, Antioxidant, Antibacterial*

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INTRODUCTION

Medicinal plants have continued to receive a lot of attention from researchers due to their pharmacological effects such as antioxidant, anti-inflammatory and antibacterial properties. The resistance to antibiotics by pathogenic microorganisms in a public health problem in the world. Indeed, many bacterial strains show resistance to several groups of antibiotics [1]. However, in the beginning of the 20th century, scientists have begun to search other molecules with antibacterial activity. Medicinal plants are natural and safer sources of anti-free radical compounds. Reactive oxygen species (ROS) provoke oxidative damage which plays a role in

the pathogenesis of diseases such as cancer, atherosclerosis and Alzheimer's [2,3].

Brassicaceae is a large family made up of vegetables (Brassica crops), medicinal plants (*Capsella bursa-pastoris*), oil-rich plants (*Brassica napus*) and the model spices of plant science (*Arabidopsis thaliana*). Among these plants, the seeds of *Lepidium sativum* (garden cress) are widely used in Arabic countries for their medicinal properties. They have various nutritional and medicinal attributes, and are recommended for anti-diarrheal [4], cardiotonic, hypotensive, antimicrobial, bronchiodilator and hypoglycemic applications [5]. These activities can be attributed to bioactive compounds as flavonoids present in this plant. Flavonoids (C6-C3-C6) can be classified into different sub-classes (flavones, flavanones, flavonols, isoflavones, flavanols, chalcones and anthocyanins) and they are commonly found conjugated to sugars in the form of O-glycosides or C-glycosides forms [6].

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EXPERIMENTAL

Plant seed collection

Seeds of *L*. *Sativum* were collected from an area in the Northwest of Algeria in May 2012. The seeds were identified by Dr H. Hadj-Arab of the laboratory of Eco-genetic and Biosystemtics, Houari Boumedien, according to the flora of Algeria. The identification was done in the herbarium of Botany Department, Ecole National Supérieure Agronomique d'Alger (ENSA), and a voucher specimen no. P12 was assigned.

Extract preparation

Twenty grams of the seeds were extracted three times with 70 % methanol for 48 h. The extract was then filtered through Whatman No.2 paper. Two more 20g batches were similarly extracted, and the combined extract was concentrated in vacuum at 40 °C. It was extracted with different solvents (50 mL each) in order: hexan, ethyl-ether, ethyl-acetate and n-butanol. Each solvent extract was evaporated and taken up in methanol. Only the n-butanol fraction was used for the identification of phytochemical composition, as well as for antimicrobial and antioxidant assays.

LC–MS analysis of flavonoids

The extract was injected into the ESI source using a Waters Acquity I-Class separation module with an Acquity PDA UV detector. Separation was achieved on an Uptisphere C18 ODB, 100 x 2.1 mm. The flow rate was 0.4 ml/min, with elution gradient of 0.1 % acetic acid (A) and 0.5 % acetic acid (B) in acetonitrile, in the order 0 – 2 min 5 % A, 4min 10 % A, 17min 40 % A, 21–23min 100 % B, 23 – 28min 5 % A. The components were identified and quantified at positive and negative ESI modes using triple quadripole mass spectrometer (Xevo TQ-S, Waters) at full scan. The operating conditions used were: capillary and extraction voltages of 2.80kVand 3V, respectively; with gas desolvation and source block temperatures fixed at 150 and 300 °C, respectively. Desolvation and nebulisation were facilated using nitrogen gas at flow rates of 200 and 1000L/h, respectively.

Determination of total phenolic contents

The levels of total phenolic compounds in the extract were quantified colorimetrically as gallic acid equivalents (GAE) with Folin-Ciocalteu reagent as described earlier [8]. The results were expressed as GAE/gram dry weight.

Determination of minimum inhibitory concentration (MIC) of extract

Antimicrobial test was carried out on five reference strains: *S. aureus* (ATCC 25923, meticillino-resistant), *S. aureus* (ATCC 43300 meticillino-sensitive), *E. feacalis* (ATCC 29212),

E. coli (ATCC 25992), and *P. aeruginosa* (ATCC27852). The MIC and IC_{50} values for the extract were determined using the serial broth dilutions method in sterile tubes [1,9,10]. The ranges of final extract concentrations used were 0.05- 32mg/mL. Each tube was inoculated with a standardized inoculum of 10^6 cells. This inoculum was obtained by diluting 2-3 well-isolated colonies on nutrient agar in 10 mL of nutrient broth. Following homogenization, the diluted colonies were placed in an incubator for 3 h at 37 °C. Thereafter, one milliliter of each culture medium was introduced into 10mL of nutrient broth in a tube containing the extract. The tubes were assessed for turbidity as evidence of bacterial growth After incubation at 37 °C for 24 h, And MIC was calculated in terms of the lowest extract concentration that produce 100% growth suppression. Unexposed organisms were used as control.

Determination of *in vitro* antioxidant activity

DPPH method

The method of Brand-Wiliams *et al* [11] was used. To 1mL of extract was added 2mL of 0.4 mM DPPH in methanol. The mixture was incubated in the dark for 30min, and the absorbance was read at 517nm. All determinations were performed in triplicate. The DPPH scavenging activity (D) was calculated using Eq 1.

D (%) = {(Ac As)/Ac}100 …… (1)

Reducing power (FRAP) method

Reducing power (FRAP) was evaluated using the potassium ferricyanide colorimetric method as described by Oyaizu [12].

β-Carotene/linoleic acid method

β-Carotene/linoleic acid spectrophotometric method was used to estimate the antioxidant activities of the extract [13]. The principle of this procedure is that the substrate β-carotene undergoes color change as a result of linoleic acid oxidation. The decrease in absorbance is measured after 120min at 470nm. In the assay, an aliquot of the extract solution (0.2mL) was mixed thoroughly with 4.8mL of linoleic acid emulsion and the absorbance was read at 470nm, with butylated hydroxyl toluene (BHT) as standard. The % inhibition of ß-carotene bleaching (B) was calculated as relative antioxidant activity (RAA) [14] using Eq 2.

$B\ (\%) = \{(A_t/A_{BHT})\}100$ …….. (2)

where A_t = absorbance of the test sample, and A_{BHT} = absorbance of the standard control (BHT)

Statistical analysis

The data are presented as mean ± SD and were analyzed by one-way analysis of variance (ANOVA) using Statistica software, followed by Tukey's multiple range tests. $P < 0.05$ was considered statistically significant.

RESULTS

Compounds identified in *Lepidium sativum* seed extract

Seventeen Compounds were identified from the seed extract of *Lepidium sativum* by LC-MS, as shown in Table 1.

These compounds included glucotropaeoline (glucosinolate), sinapoyl malate, sinapic acid, sinapine, sinapoyl diglucose and ferulic acid., Glucotropaeoline was the most abundant component (67.5 %) followed by sinapine (13.43 %), In addition, *Lepidium sativum* contained relatively high concentrations of flavonoids. From the results obtained, quercetin and kaempferol were the main flavonols present, with varying degrees of glycosylation (mono, di and tri-glycoside). Many isomers of hexose rhamnoside and other derivatives were also present. All the glycosides were attached to a rhamnose.

Four components (heterosides) were identified for the first time. According to their HPLC retention times and MS profiles, these heterosides were identified as two isomers of kaempferol hexose rhamnose (ion at *m/z* 595 $[M-H]^+$) and three derivatives of kaempferol rhamnose hexose (benzoyl-hexose) (ion at 861 *m/z* $[M-H]^+$). The other compounds have been previously identified [15].

Kaempferol di-hexoside rhamnose and quercetin di-hexoside rhamnose were the most important heterosides present in *L. sativum* (at high levels 3.77 and 2.07 %, respectively).

Antioxidant activity and total phenolic contents of the extract

The results are presented in Table 2. The IC_{50} of the n-butanol extract in the DPPH radical scavenging assay was 67.1±0.3 µg/mL. Reducing power (FRAP) and % inhibition of β-carotene bleaching were 100±0.05 $\mu g.mL^{-1}$ and 54.91%, respectively. The antioxidant activity of the butanolic extract was < the standards (ascorbic acid, quercetin and α-tocopherol).

Table 1: Quantitative and qualitative characterization of phytochemical compounds from *L. sativum*

Compound	RT	Molecular ion MH-	Molecular ion MH+	Proportion crude extract	Compound (%)
Glucopaoline	-3.43	408, 410	/	187.12	67.507
Sinapoyl malate	-7.73	339, 223	207	4.62	1.67
Ferulic acid	-7.95	193	195	0.38	0.137
Sinapic acid	-8.15	223	225	1.53	0.552
Sinapoyl di-glucose	-12.9	573,223	575	7.41	2.675
Sinapine	+5.97	/	310	37.2	13.43
Q di-hexose rhamnose	+5.99	771	773	5.73	2.07
K di-hexose rhamnose	+6.4	755	757	10.46	3.77
K hexose rhamnose 1	+7.15	/	595	4.68	1.69
Q hexose rhamnose	+7.35	609	611	0.17	0.06
K hexose rhamnose 2	+7.75	593	595	0.26	0.837
K rhamnose (benzo) di-hexose 1	+8.84	859	861	2.96	1.867
K rhamnose (benzo) di-hexose 2	+9.25	859	861	0.31	0.11
K hexose rhamnose 3	+10.19	/	595	1.57	0.566
Q rhamnose	+10.94	447	449	0.51	0.322
K rhamnose (benzo) di-hexose 3	+11.01	859	861	0.98	0.35
K rhamnose	+12.45	431	433	0.29	0.1

Q = quercetin, K = kaempferol

Table 2: Total phenolic content expressed as mg/g gallic acid equivalent (EAG), and antioxidant activity of n-butanol extract *L.sativum* seeds

	Total polyphenols mg/gEAG	Antioxidant activity		
Material		DPPH (IC_{50}(µg.mL^{-1})	RP (µg.mL^{-1})	β–Caroten(%)
Extract	1.629±0.13	67.1±0.3*	100±0.051	54.91±0.116*
Ascorbic acid	-	4.04±0.13*	7.53±0.104*	30.55±0.117*
Quercetin	-	6.72±0.158*	20±0.01*	45.37±0.12**
α-Tocopherol	-	-	-	98.55±0.248*

Values (mean ± SD, n = 3); *significant difference ($p < 0.05$)

Table 3: Antibacterial activity of n-butanol extract *L. sativum* seeds

Parameter	Antibacterial activity (mg.mL^{-1})				
	E. coli	*P. aeruginosa*	*S. aureus 1*	*S. aureus 2*	*E. faecalis*
IC_{50}	0.41	0,42	0,47	0,42	0,4
MIC	5	4	4	4	3,5

Antibacterial activity

The results of antibacterial activity of *Lepidium sativum* which was evaluated *in vitro* against five bacterial strains Table 2. The calculated MIC and IC_{50} are presented in the Table 3. The n-butanol fraction has exhibited appreciable antibacterial activity against all the bacteria strains tested irrespective of Gram strain.

DISCUSSION

The main flavonols present in many Brassicaceae species are quercetin, kaempferol and their derivatives (mono-, di-, tri-glycosides) [16,17]. This is in agreement with the results obtained in the present study. A previously unknown quercetin-3-*O*-(6-*O*-benzoyl)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside-7-*O*-α-L rhamnopyranoside was identified by Fan *et al* [7]. However, while the same acylation was present in the Algerian variety, quercetin was replaced by a kaempferol. The results available in the literature regarding the phytochemical compositions of leaves of *Lepidium sativum* and leaves of other Brassicaceae plants indicate the presence of three flavonols viz quercetin, kaempferol and isorhamnetin [18]. Thus, it seems that isorhamnetin is absent in the seeds.

Results from various studies indicate that all flavonoids glycosides possess strong antimicrobial activity against strains of *P. aerugenosa* and *S. aureus* but exhibit a very low activity against *E. coli* [19]. . This is also in agreement with the results of antimicrobial activity of the n-butanol extract used in this study. Studies on methanol and chloroform extract of *Lepidium sativum* have also shown low antibacterial activity against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* [20]. The antibacterial activity of

flavonol glycosides towards Gram-positive bacteria may be related to the cell membrane constituents and structures of these organisms. The flavonols may also be inhibitory to the activities of cellular enzymes. It has been also reported that flavonol glycosides chelate metals, an effect which can inhibit some enzymes [21]. It is important to mention that the permeability of the bacterial cells to the tested compounds is one of the factors that determine their antibacterial effects.

The flavonols have the ability to donate hydrogen ions to synthetic free radical compound (DPPH) [22], and to reduce potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}) by donating an electron [23]. These antioxidants can stop the conversion of linoleic hydro-peroxide or eliminate this peroxide [24]. The differential activities of flavonoids could depend on the solvent used for extraction and on the position of substituents in the flavonoid nucleus. These differences in substituents give rise to the major bioactive compounds such as quercetine, kampferol and their glycosides which are present in *Lepidium sativum* seeds [7,15-17].

CONCLUSION

The results of this study indicate that n-butanol extract of *L. sativum* seed possesses significant antioxidant properties, and significant antibacterial activities against selected Gram-positive and Gram-negative pathogens. Further investigations are needed to elucidate the structures of the identified compounds in detail using nuclear magnetic resonance (NMR). It would also be helpful to carry out *in vivo* tests on these compounds in their isolated and purified forms.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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