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Asian Pacific Journal of Tropical Disease

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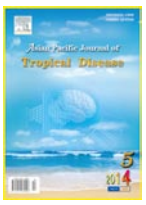

Document heading doi:10.1016/S2222-1808(14)60587-9 © 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Assessment of *in vitro* antimicrobial potency and free radical scavenging capacity of the essential oil and ethanol extract of *Calycotome villosa* subsp. intermedia growing in Algeria

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PEER REVIEW

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Comments

This is an interesting study which confirms the potency of *C. villosa* essential oils and ethanol extract as effective antibacterial agents. In fact, tested on strong resistant bacteria which are responsible for nosocomial disease, the extracts prepared show a high activity.
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ABSTRACT

Objective: To assess the antimicrobial and antioxidant activity of the essential oil and ethanol extract of the aerial parts of *Calycotome villosa* subsp. intermedia growing in the West Northern region of Algeria.

Methods: Chemical composition of essential oils obtained by hydrodistillation from areal parts of *Calycotome villosa* subsp. intermedia was investigated using gas chromatography (retention indices) and gas chromatography-mass spectrometry while the antimicrobial activities were determinate by paper disc diffusion method and minimum inhibitory concentration assays tested against four bacterial strains and one yeast and antioxidant activity was evaluated as a free radical scavenging capacity (RSC).

Results: Essential oils were dominated by non-terpenic compounds and fatty acids. However, the phenylpropanoids, monoterpenes and sesquiterpenes components were only present in small percentages. The most important antibacterial activity of essential oil was expressed on *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Antioxidant activity was evaluated as a RSC. RSC was assessed by measuring the scavenging activity of essential oil and ethanol extract on 1,1-diphenyl-2-picrylhydrazil (DPPH). Investigated ethanol extract reduced the DPPH radical formation (IC_{50}=68 μg/mL).

Conclusions: Results in this experiment indicate that the essential oil and the ethanol extract display antibacterial activity against two Gram-positive bacteria and activity to a lesser extent against two Gram-negative species. They may be a new potential source of components, which are likely to have impact on human health.

KEYWORDS

Calycotome villosa subsp. intermedia, Essential oil, Fatty acid, Antimicrobial activity, Radical scavenging capacity

1. Introduction

The role of natural antioxidants and antimicrobial in disease prevention and treatment has gained interest by the health community. However, plant volatile essential oils (EOs) and solvent extracts from a wide variety of

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Foundation Project: supported by the University of Tlemcen and the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic under the project PNR (Grant NO. 267/ANDRS/2011-2013).

Article history:
Received 10 May 2014
Received in revised form 19 May, 2nd revised form 23 May, 3rd revised form 28 May 2014
Accepted 16 Jun 2014
Available online 3 Jul 2014

plants have been assessed. It is clear from these studies that these secondary plant metabolites possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities[1,2]. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[3,4]. The use of secondary plant metabolites in medical procedures and applications in the cosmetic, pharmaceutical and food industries is crucial. The antimicrobial activities of EOs have been well recognized for many years[5]. This activity could act as a chemical defence against plant pathogenic diseases. The development of bacterial resistance to presently available antibiotics necessitated the search for new antimicrobial agents. *Calycotome* is a genus of flowering plants in the legume family, Fabaceae. It belongs to the sub family Faboideae. It may be synonymous with *Cytisus*. *Calycotome villosa* (*C. villosa*) is a hairy perennial with yellow flower heads in branched clusters; found almost everywhere on the Algerian coast[6]. However, no uses in the folk medicine are known for this species. Phytochemical studies have revealed that the plant is rich in flavonoids, terpenes, alkaloids, falcarinol and anthraquinones[7]. However, very little research has been carried out on EO of the aerial part from *C. villosa*. The chemical composition of EO reported in literature was studied partially[7,8]. Tests of the cytotoxic and antifungal activity exhibited by *C. villosa* have also been assessed[8]. The methanol extract and the EO from *C. villosa* were non toxic (LD_{50}>500 μg/mL) and inhibited the growth of *Staphylococcus aureus* (*S. aureus*), *Barosaurus lentus* and *Cryptococcus neoformans* at a dose of 500 μg/mL. On the other hand, the cytotoxic activity of EO and crude methanol extract has also been evaluated and that showed strong cytotoxicity at 0.04 μL/mL[7], mainly due of presence of falcarinol which possesses some negative effects and toxic in relatively high concentration[9,10]. However, it has been quite recently reported that some polyacetylenes, in particular falcarinol, isolated from carrots have been reported as having a beneficial effect on human health[11]. Therefore, this study was undertaken in order to: (i) determine the full chemical composition of EO extracted by hydrodistillation using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), (ii) investigate the effectiveness "*in vitro*" on survival and growth of selected foodborne pathogens using agar disc diffusion and broth microdilution methods, and (iii) assess the antioxydant activity by means of radical scavenging capacity (RSC) of ethanol extract and EO of aerial parts from *C. villosa*.

2. Materials and methods

2.1. Plant samples

For this study, fresh plant material (aerial parts: stems, leaves and flowers) was used. The plant was collected on March 2011, in Mansourah (S1, Latitude: 34°52′15.56″N, Longitude: 1°20′20.71″W, Altitude: 821 m) and Terny (S2, Latitude: 34°46′58.8″N, Longitude: 1°18′W, Altitude: 1525 m) forests near Tlemcen, Algeria. The botanical identity of the plant material was confirmed by Pr Benabadji Nouri and the voucher specimens are deposited at Laboratory of Ecology and Ecosystem Management, University of Tlemcen (Algeria).

2.2. Essential oil

Plant material (200 g) was subjected to hydrodistillation (in 4000 mL distilled water) for 5 h, using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia[12]. The EO yield was 0.01% for (S1) and 0.02% for (S2) (w/w). The EOs were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4-5 °C prior to analysis.

2.3. Preparation of the ethanol extract

The air-dried and powdered plant material (10 g) was extracted with 200 mL of ethanol by Soxhlet extraction for 4 h. The obtained ethanol extract was filtered and evaporated by using a rotary evaporator and freeze dryer (yield: 35.1%). The dried extract was stored at − 20 °C until tested.

2.4. GC analysis

GC analyses were carried out using a Perkin-Elmer (Waltham, MA, USA) Autosystem XL GC apparatus equipped with a dual flame ionization detection system and a fused-silica capillary columns (60 m×0.22 mm id, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50), using helium as the carrier gas (1 mL/min); the injection volume was 0.2 μL. Retention indices (RI) of the compounds were determined relative to the retention times of the series of n-alkanes (C_5-C_{30}) with linear interpolation, using the Van den Dool and Kratz equation and software from Perkin-Elmer. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.5. GC/MS analysis

Samples were analyzed with a Perkin-Elmer Turbomass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, and equipped with the fused-silica capillary columns Rtx-1 (ion source temperature 150 °C; energy ionization 70 eV). EI mass spectra were acquired over the mass range 35-350 Da (scan time: 1 second). Other GC conditions were the same as described under GC except split 1/80.

2.6. Component identification

Identification of individual components was based (i) on comparison of calculated RI, on polar and apolar columns, with those of authentic compounds or literature data, and (ii) on computer matching with commercial mass spectral libraries and comparison of mass spectra with those of our own library of authentic compounds or literature data[13-16].

2.7. Antimicrobial activity

Tests were performed against four bacteria reference strains: Gram-positive bacteria: *Enterococcus faecalis* ATCC 49452 (*E. faecalis*), *S. aureus* ATCC 25923, Gram-negative bacteria: *Klebsiella pneumoniae* ATCC 70063 (*K. pneumoniae*), *Salmonella typhimurium* ATCC 13311 (*S. typhimurium*) and a yeast: *Candida albicans* ATCC 14053 (*C. albicans*). The microorganisms were obtained from Pasteur Institute of Paris. Two different techniques were used to test the antimicrobial activity: the paper disc diffusion and the dilution agar method. The minimum inhibitory concentration (MIC) was determined by the later method.

2.7.1. Paper-disc diffusion method

Filter paper discs (6 mm in diameter) were individually impregnated with 10 μL of essential oil and 5 μL of ethanol extract (saturated with a 50 mg/mL) and placed on the inoculated plates and, after staying at 4 °C for 2 h, they were incubated at 37 °C for 18-24 h for bacteria, at 35 °C for 48 h for the yeast. The plates were incubated overnight at the appropriate temperature (see above), and the diameter of the resulting zone of inhibition was measured in millimeters. The results in the text represent the net zone of inhibition including the diameter (6 mm) of the paper disc. The scale of measurement was the following[17](disc diameter included): ⩾20 mm: zone of inhibition is strongly inhibitory; <20-12 mm: zone of inhibition is moderately/mildly inhibitory; and <12 mm is no inhibitory. All the data collected for each assay are the averages of three determinations.

2.7.2. Dilution agar method

A dilution agar method was used to determine the MIC. Stock solutions were obtained by dissolving extracts in dimethylsulfoxide (DMSO 1%). Serial dilutions were made to obtain concentrations ranging from 0 to 50 000 μg/mL of ethanol extract and EO. Each mixture was added to Mueller-Hinton agar for bacteria and Sabouraud dextrose agar with Chloramphenicol for yeast (cooled to 45-50 °C[18,19]. Two controls were included in this test. The Petri dishes contained a sterile solution of DMSO and the culture medium, respectively. The experiments were performed in triplicate. After incubation at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeast.

2.8. RSC

The hydrogen-donating abilities of the tested extracts were examined on the basis of the method described in literature with some modifications[20,21]. Used as reagent, DPPH obviously offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. Fifty microliters of various concentrations of the EO and ethanol extract were added to 5 mL of a 0.005% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of the RSC and DPPH in percent (I %) was calculated in the following way:

$I\ (\%)=100\times(A_0-A_s)/A_0$

Where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the tested sample.

The actual decrease in absorbance induced by the tested sample (change of color from deep-violet to light yellow) was compared to that of the positive control ascorbic acid. The IC_{50} value represented the concentration of extract that causes 50% inhibition was determined. Experiments were carried out in triplicate and the mean value was recorded.

3. Results

3.1. GC/MS analysis of EO

The results of both analyses are shown in Table 1. Fifty compounds were identified by GC and GC/MS, which accounted for 91.1%-91.8% of the total composition. The EO was complex mixture of non-terpenic compounds, monoterpenes and sesquiterpenes. The non-terpenic compounds made up the higher contribution (65.0%-65.7%) in the EO of *C. villosa*. The content of four aliphatic compounds (heneicosane, tricosane, pentacosane and heptacosane) amounted to 39.6%-43.8%. The fatty acids (14.0%-16.7%) were found as the second main chemical classes. The GC/MS enabled the identification of five acids. Pentadecanoic acid (10.3%-12.3%) and hexadecanoic acid (3.5%-4.0%) were the main compounds which had anti-thrombus, could prevent cardiovascular disease and has the antibacterial and antifungal activities, followed by dodecanoic acid (0.2%-0.3%) with the antibacterial and antifungal activities, nonanoic acid (0.1%-0.2%) and octadecanoic acid (trace-0.1%) with the antibacterial and antifungal activities, (Tables 1 and 2). However, Z-isoeugenol (2.4%-2.9%) was found as the major phenylpropanoid. Oxygenated compounds both mono- and sesquiterpenes were less represented. In this fraction, linalool (0.4%-1.0%), β-ionone (0.3%-0.6%), 1,8-cineole (trace-0.4%), L-fenchone (0.2%-0.4%), borneol (0.4%) and camphor (trace-0.1%) were the main oxygenated monoterpenes. E-β-damascenone (1.9%-3.7%), Z-jasmone (0.5%-0.6%) and Z-falcarinol (0.2%-0.4%) were the major

oxygenated sesquiterpenes. Other hand the mono- and sesquiterpene hydrocarbons compounds were represented by a single compound, the limonene (0.6%-1.4%) and α-cubebene (trace-0.9%) respectively (Table 1).

Table 1

Chemical composition of EO of *C. villosa*.

No[a]	Components	RI_a[b]	RI_a[c]	(S1)[d]	(S2)[d]	Identification
1	E-2-hexenal	832	832	trace	0.5	RI, MS, Ref_1
2	E-2-heptenal	927	924	0.6	0.1	RI, MS, Ref_2
3	Benzaldehyde	933	932	0.2	-	RI, MS
4	1,8-Cineole	1022	1026	0.4	trace	RI, MS
5	Limonene	1025	1031	0.6	1.4	RI, MS
6	Acetophenone	1033	1034	0.3	0.4	RI, MS, Ref_2
7	L-fenchone	1066	1059	0.2	0.4	RI, MS, Ref_2
8	Nonanal	1086	1087	0.4	1.8	RI, MS
9	Linalool	1087	1089	0.4	1.0	RI, MS
10	Camphor	1125	1131	0.1	trace	RI, MS
11	Borneol	1148	1144	0.4	0.4	RI, MS
12	Dodecane	1200	1198	0.1	0.4	RI, MS
13	Nonanoic acid	1263	1267	0.2	0.1	RI, MS, Ref_2
14	Undecan-2-one	1274	1273	0.4	0.2	RI, MS
15	Undecanal	1286	1286	0.2	0.3	RI, MS
16	(E,E)-2,4-Decadienal	1288	1291	0.4	0.5	RI, MS
17	Tridecane	1300	1301	0.4	0.8	RI, MS
18	α-Cubebene	1355	1353	0.9	trace	RI, MS
19	*E*-β-damascenone	1362	1361	1.9	3.7	RI, MS
20	Z -isoeugenol	1370	1367	2.4	2.9	RI, MS
21	Z-jasmone	1373	1379	0.6	0.5	RI, MS, Ref_2
22	Dodecanal	1384	1386	0.2	0.1	RI, MS
23	Tetradecane	1400	1399	0.8	1.0	RI, MS
24	β-Ionone	1463	1468	0.6	0.3	RI, MS
25	Tridecan-2-one	1479	1473	6.8	5.6	RI, MS
26	Tridecanal	1486	1484	0.2	0.4	RI, MS
27	Pentadecane	1500	1499	0.7	0.3	RI, MS
28	Dodecanoic acid	1556	1555	0.3	0.2	RI, MS, Ref_2
29	Hexadecane	1600	1597	0.2	0.3	RI, MS
30	Pentadecan-2-one	1682	1684	7.5	4.2	RI, MS
31	Pentadecanal	1694	1693	1.5	0.5	RI, MS
32	Heptadecane	1700	1698	0.2	0.6	RI, MS
33	Benzyle benzoate	1730	1726	0.7	0.2	RI, MS, Ref_2
34	Octadecane	1800	1800	trace	0.3	RI, MS
35	Pentadecanoic acid	1839	1836	12.3	10.3	RI, MS, Ref_2
36	Heptadecan-2-one	1882	1883	0.6	0.5	RI, MS, Ref_2
37	Nonadecane	1900	1898	0.3	0.4	RI, MS
38	Hexadecanoic acid	1951	1956	4.0	3.5	RI, MS, Ref_2
39	Z-falcarinol	1994	1998	0.4	0.2	RI, MS
40	Eicosane	2000	2000	0.7	0.4	RI, MS
41	Octadecanol	2078	2074	0.2	0.1	RI, MS
42	Heneicosane	2100	2102	6.4	15.6	RI, MS
43	Octadecanoic acid	2162	2169	0.1	trace	RI, MS, Ref_2
44	Docosane	2200	2199	0.7	0.9	RI, MS
45	Tricosane	2300	2302	15.2	17.7	RI, MS
46	Tetracosane	2400	2400	1.4	0.9	RI, MS
47	Pentacosane	2500	2501	12.3	6.4	RI, MS
48	Hexacosane	2600	2600	0.5	0.4	RI, MS
49	Heptacosane	2700	2700	5.7	4.1	RI, MS
50	Octacosane	2800	2799	0.4	0.4	RI, MS
Total identification %				91.8	91.1	
Monoterpene hydrocarbons				0.6	1.4	
Oxygenated monoterpenes				2.1	2.3	
Sesquiterpene hydrocarbons				0.9	-	
Oxygenated sesquiterpenes				2.9	4.2	
Phenylpropanoids				3.6	3.5	
Fatty acids				16.7	14.0	
Non-terpenic compounds				65.0	65.7	

[a]: order of elution is given on apolar column (Rtx-1); [b]: retention indices of literature on the apolar column (RI_a); [c]: retention indices on the apolar Rtx-1 column (RIa); [d]: percentage (%); S1: Mansourah; S2: Terny; RI: retention indices; MS: mass spectrometry in electronic impact mode; Ref_1: compounds identified from literature data[13]; Ref_2: compounds identified from literature data[14].

3.2. Antimicrobial activity

3.2.1 Paper disc diffusion method

The *in vitro* antibacterial activities of EO and ethanol extracts of *C. villosa* against the employed bacteria were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. The results of the bioassay are shown in Table 3. The inhibition zone, measured in millimeters, including the diameter of the paper disc, was used as the criterion for measuring the antibacterial activity of *C. villosa* EO and ethanol extract. Table 3 shows that the tested extracts could be classified according to their activity. The high activity extract of this plant was EO with an antimicrobial inhibition zone of 14 and 13 mm against *S. aureus* and *E. faecalis* respectively and lowly activity against *K. pneumoniae* and *S. typhimurium* with an antimicrobial inhibition zone of 11 mm. The ethanol extract had average activity against two food poisonous bacteria: *S. aureus* and *S. typhimurium*, with inhibition zone of 10 mm. However, *C. albicans* strain showed no inhibition, with diameter of zones of inhibition equal to 6 mm (Table 3).

Table 2

Saturated and unsaturated fatty acid identified in aerial part of *C. villosa*.

Acid components	Mol. Formula	Mol. weight
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4
Dodecanoic acid	$C_{12}H_{24}O_2$	200.3
Octadecanoic acid	$C_{18}H_{36}O_2$	284.5
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4
Nonanoic acid	$C_9H_{18}O_2$	158.2

Table 3

Antimicrobial activity of EO and ethanol extract from *C. villosa*.

Species	Disc diffusion assay (mm)		MIC (μg/mL)	
	EO	Ethanol extract	EO	Ethanol extract
Gram-positive bacterium				
S. aureus	14	10	30	21 250
E. faecalis	13	7	60	42 500
Gram-negative bacterium				
K. pneumoniae	11	8	120	42 500
S. typhimurium	11	10	120	21 250
Yeast				
C. albicans	6	6	>50 000	>50 000

3.2.2. MIC

The antimicrobial activity of the investigated EO and ethanol extract was evaluated by determining MIC values against two Gram-positive and two Gram-negative bacteria as well as against one fungal strain. The results exhibited that the EO had varying degrees of growth inhibition against the bacterial strains (Table 3). However, no activity was registered against *C. albicans*. The Gram-positive strains showed more susceptibility to the tested EO than the Gram-negative ones. The most prominent inhibitory

action of EO was observed against *S. aureus* and *E. faecalis* with a MIC of 30 and 60 μg/mL respectively. However, *K. pneumoniae* and *S. typhimurium* showed low activity with MIC values of 120 μg/mL. Furthermore, the ethanol extract of *C. villosa* also shows antimicrobial properties with MIC values ranging from 21.2 to 42.5 mg/mL (Table 3).

3.3. RSC

In order to assess the RSC potential of the EO and ethanol extract, the reactivity towards the stable free radical DPPH was measured. DPPH is one of the chemical compounds that possess a proton free radical and it shows a maximum absorption at 517 nm because of its bright purple color. When DPPH encounters proton radical, its purple color fades rapidly and this scavenging action forms the basic mechanism for measuring antioxidant activity. Table 4 demonstrates DPPH scavenging activity, expressed in percentage, caused by different concentrations of EO and ethanol extract from *C. villosa*. The weakest RSC was exhibited by the EO (60%), whereas the strongest activity was exhibited by the ethanol extract (96%) at a concentration of 200 μg/mL, comparing with the effect of ascorbic acid at this concentration (Table 4).

Table 4

DPPH radical-scavenging of EO and ethanol extract from *C. villosa* at different concentrations.

Source	Concentrations (μg/mL)	Inhibition (%)	IC_{50} values (μg/mL)
Ethanol extract	50	30.00	
	80	52.00	68
	100	92.00	
	200	96.00	
Essential oil	100	6.00	
	400	13.00	1 250
	800	42.00	
	1 600	60.00	
Ascorbic acid	40	38.32	
	80	96.16	47
	100	98.02	
	200	98.61	

4. Discussion

The EO of *C. villosa* contains the non-terpenic compounds and fatty acids as the main component, followed by phenylpropanoids and oxygenated sesquiterpenes. The EO was relatively poor in monoterpenes. When the chemical composition of the EO of the studied plant was compared to that obtained for *C. villosa* gathered in Italy[7], we noted a significant difference in the percentages of its constituents and number of molecules identified. The results of the study indicated that EO of *C. villosa* exerted higher antibacterial activity than ethanol extract. Regarding to the composition of the EO of *C. villosa*, various chemical compounds isolated by hydrodistillation have direct activity against many species of bacteria. Oxygenated monoterpenes such as camphor, borneol and linalool, were reported to be responsible for the antimicrobial activity of several EOs[22]. However, phenylpropanoids are highly active against a broad spectrum of microorganisms[23,24]. The importance of the hydroxyl group has been confirmed and the relative position of the hydroxyl group on the phenolic ring does not appear to strongly influence the degree of antibacterial activity[25,26]. Moreover, the predominance of fatty acids (14.0%-16.7%) could probably contribute to the observed activity, since many fatty acids are known to have antibacterial and antifungal properties[27]. Furthermore, polyacetylenes, in particular falcarinol taken in small quantities has been reported as having a beneficial effect on human health[10,28]. Recent reports on ginseng roots have showed antimutagenic properties and stimulatory effects on the immune system during bacterial infection probably due to the presence of falcarinol and related polyacetylenes. Consequently, the antibacterial activity of *C. villosa* could be attributed to the presence of many compounds such as, oxygenated monoterpenes, phenylpropanoids, fatty acids and falcarinol or to the synergistic effect between these compounds. Regarding RSC activity, it was observed that the ethanol extract of *C. villosa* showed a significant dose dependent inhibition of DPPH radical scavenging activity compared to EO. The extract exhibited a noticeable activity at low concentrations. This suggests that extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. According to these results, there is a relationship between chemical composition and antioxidant activity. Moreover, as reported in literature data, the antioxidant activity of ethanol extract could be attributed to phenolic compounds, flavonoids and alkaloids[29].

In conclusion, the GC/MS analysis of the EO in the aerial parts of *C. villosa* revealed that the chemical compositions obtained differed from that investigated previously. The *C. villosa* was rich in non-terpenic compounds and fatty acids with tricosane, pentacosane, pentadecanoic acid, heneicosane, pentadecan-2-one and tridecan-2-one as major components. Hexadecanoic acid is

the main compounds which has anti-thrombus, can prevent cardiovascular disease and has the antibacterial and antifungal activities[27,30-32]. Dodecanoic acid and octadecanoic acid have the antibacterial and antifungal activities[27,31,32]. This oil exhibited the best antibacterial activity against *S. aureus*, *E. faecalis*, *K. pneumoniae* and *S. typhimurium* bacterias. However, the ethanol extract exhibited a different range of RSC. The components responsible for the antioxidant activities of the extract were not identified and further studies are in progress in our laboratory to isolate the active components of *C. villosa* responsible for this activity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

I wish to thank Professor N. Benabadji of the Botanical Laboratory, Biology Department, Abou Bekr Belkaïd University for the botanical identification of the *Fabaceae* obtained from numerous sources. This work was financially supported by the University of Tlemcen and the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic under the project PNR (Grant. No. 267/ANDRS/2011-2013).

Comments

Background

C. villosa is widely distributed in Algeria. Tests of the cytotoxic and antifungal activity exhibited by *C. villosa* have been assessed. Since detailed *in vitro* antimicrobial and antioxidant activities of *C. villosa* aerial parts have not yet been explored, the authors undertook a detailed investigation in order to determine the full chemical composition of essential oil, to investigate the effectiveness *in vitro* on survival and growth of selected foodborne pathogens, and to screen antioxidant activity of both ethanol extract and essential oil.

Research frontiers

This study is to ascertain the antibacterial and antioxidant effects of *C. villosa* essential oils and ethanol extract against ascorbic acid.

Related reports

Earlier works by Loy *et al*., 2001 and Dessí *et al*., 2001, recorded comparable antioxidant and antibacterial effects of essential oils and alcoholic extracts.

Innovations & breakthroughs

The paper provides information on the efficacy of *C. villosa* essential oils as antibacterial agent against Gram-positive bacteria: *E. faecalis*, *S. aureus*, which account for the majority of episodes of bacteremia in critically ill patients in the hospital environment: the high level of natural resistance to antibiotics of these bacteria contributes to its pathogenicity and nosocomial risk.

Applications

The result of this study confirms *C. villosa* as a formidable source of interesting components, which are likely to have impact on human health.

Peer review

This is an interesting study which confirms the potency of *C. villosa* essential oils and ethanol extract as effective antibacterial agents. In fact, tested on strong resistant bacteria which are responsible for nosocomial disease, the extracts prepared show a high activity.

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