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UPLC-MS profiling, antimicrobial and antipyretic activities of *Deverra scoparia* Coss. & Dur. extracts

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Deverra scoparia Coss. & Dur. is a traditional medicinal plant with important biological properties, due to the presence of secondary metabolites. This study aimed to evaluate antimicrobial and antipyretic activities of infusion and ethereal extracts. The antimicrobial activity was tested using disk diffusion method against bacterial and fungal strains. While the antipyretic activity was assessed by yeast induced pyrexia in rats at doses of 250, 500 and 1000 mg/kg for each extract. Results showed a strong antimicrobial activity range by the extracts; the infusion extract has an increased inhibition from 45±0.004 mm and 52±0.003 mm with bactericidal effect against all bacterial strains tested. The ethereal extract presented a strong antimicrobial activity about 32±0.001 mm and 48±0.001 mm with bacteriostatic effect with a value equal to 8 for all strains. The administration of infusion and ethereal extracts produced significant ($p<0.05$) and dose-dependent decrease in rectal temperature. The UPLC-MS analysis indicated the presence of caffeic acid and quercetin with a quantity of 16.4 and 1224.6 µg/g sample respectively. These findings indicate that *Deverra scoparia* Coss. & Dur. aerial part contains compounds with antimicrobial and antipyretic properties, thus justifying its use in traditional medicine.

Keywords: Antimicrobial, Antipyretic, Caffeic acid, *Deverra scoparia*, Quercetin

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Since antiquity, plants have been regarded as a source of medicinal agents due to their therapeutic properties. These plants have been used extensively in traditional medicine to treat various diseases and some of these are still included as part of the habitual treatment of various maladies[1]. It was estimated that one quarter of approved modern medicines are directly or indirectly derived from plants[2,3]. Recent emergence of antibiotic resistance and the various side effects of antimicrobial drugs have led people to turn to traditional remedies as an alternative[4], such as plants which contain antimicrobial compounds that can potentially be effective in the treatment of these bacterial infections[5]. Many countries encouraging the screening programs of herbs from traditional medicine in order to authenticate their pharmacological preperties and the possible including in the primary health care[6]. In Algeria phytotherapy is practiced by a great proportion of the Saharan population, particularly Touareg for the treatment of several ailments. The Apiaceae or Umbelliferae is represented in Algeria with 55 genera, 117 species, 24 of which are endemic as *Deverra scoparia* Coss. & Dur. This species is an endemic plant which grown in rocky pastures of North Africa region and widespread in most regions of the Algerian Sahara[7]. In Algerian traditional medicine, the aerial parts of *Deverra scoparia* Coss. & Dur. are used in the treatment of several infectious diseases, rheumatism, pains, fevers, digestive difficulties and urinary infections[8,9]. The plant was reported to contain flavonoids and terpenoids compounds[10]. Many studies show that *Deverra scoparia* Coss. & Dur. essential oil possesses acaricidal, antioxidant, antibacterial properties[11,12]. According to literature data, not present research on these activities for this plant extracts. This study aimed to evaluate the antimicrobial and antipyretic activities of infusion and ethereal *Deverra scoparia* Coss. & Dur extracts. Then, the molecules which could be responsible for these biological properties were identified and quantified by Ultra Performance Liquid Chromatography-MS (UPLC-MS).

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Materials and Methods

Plant material

The material used consists of the aerial part of *Deverra scoparia* Coss. & Dur., from Tamanrasset (South of Algeria), collected in March 2017. The botanical identification of this species was carried out by the botanists of the research station on the protection of the arid regions of Tamanrasset according on the flora of Quezel and Santa[9]. A voucher specimen (N°38) has been deposited at the herbarium of the National Institute of Agronomy, Algeria. The plant material was dried, crushed and preserved from light and moisture.

Animals

Wistar albino rats of both sexes (150-200 g) were used for the experimental study. The animals were obtained from Animal Laboratory of Biotic Unit (Saidal, Algeria). They were kept in cages and acclimatised at least 5 days before experiments to controlled lab conditions of light (12-h light/dark cycle), temperature (20 – 24°C) and humidity (50–65%). Food and water were provided *ad libitum*. The experimental procedures were approved by the Natioanl Research Council Academies[13].

Microbial strains

The antimicrobial potential of extracts was assessed against two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC27853*, Escherichia coli* ATCC25922) and one Gram- positive bacteria (*Staphylococcus aureus* ATCC25923). Also, two fungal strains (*Candida albicans* ATCC24433*, Candida dubliniensis* ATCC44508). Microbial strains were grown overnight using Mueller-Hinton agar at 37°C for bacterial cultures and Sabouraud medium for fungal cultures at 25°C. Microbial stock and nutrient media were obtained from the microbiology laboratory at Mustapha Bacha Hospital (Algiers, Algeria).

Plant extracts preparation

One gram of aerial parts powder of *Deverra scoparia* Coss. & Dur. were macerated with 30 mL diethyl ether for 24 h. Thereafter, the ethereal extract was decanted and filtered, then was left to dry to remove all traces of the solvent. After that, ethereal extract was recuperated in 3 mL methanol. The infusion extract was prepared by adding boiling distilled water to the sample (1 g / 50 mL) and were left to stand at room temperature for 10 min and then filtered. The ethereal and infusion extracts were used for biological tests. For the extraction of phenolic compounds, 250 mg of aerial part powder were extracted with 5 mL of acidified (0.1% v/v acetic acid) methanol/water (50:50) solution and twice with 5 mL of acetone: water (70:30) during 30 min in an ultrasonic bath. The extracted solution was diluted with 1:4 acidified (acetic acid at 0.1%) Milli-Q-water, then filtered using 0.20 µm PTFE filters. The phenolic compounds extract was conserved at 6°C for the UPLC-MS analysis. The solvents which used in the extraction contains ascorbic acid (0.2% w/v)[14].

Antimicrobial activity

The antibacterial activity of infusion extract was investigated using disk diffusion method according to Sacchetti *et al.*[15]. The concentration of the cell suspension was adjusted to the 0.5 McFarland standards, and 50 µL of microbial inoculum was spread on an agar plate. Dried filter paper discs (9 mm in diameter), previously impregnated with 100 µL of ethereal and infusion extracts and placed on the surface of agar plate. The positive controls used are Vancomycin and Gentamicin at 10 µg/mL respectively for positive and negative Gram bacterial strains. Miconazole (10 µg/mL) disc were used as positive control for fungal strains. Sterile water, diethyl ether and methanol were used as negative control. The plates were incubated for 24 h at 37°C for bacterial cultures and at 25°C for fungal cultures. The experiment was done in triplicate. The results were expressed by measured the diameters of the inhibition zones in millimeter then compared with positive control to classify the microbial strain as susceptible, intermediate or resistant[16].

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was carried out using the method of Ganière *et al.*[17]. Serial dilutions of ethereal and infusion extracts (0.25–64 mg/mL) were prepared and performed in duplicate. One column was used for sterility control and another one for growth control. Each bacterial suspension was diluted to 5×10^5 CFU/mL with 50 µL of Mueller-Hinton broth and the sterility controls were prepared by using Mueller-Hinton broth alone. Then, incubation of plate at 37°C for 24 h and the minimum inhibitory concentration was reported as the lowest concentration that could inhibits the bacterial growth.

Determination of minimum bactericidal concentration (MBC)

Minimum bactericidal concentration was determined by the method of CLSI[18], aliquots of 100 µL from each well with no visible bacterial

growth (starting with MIC tube) was streaked in Mueller-Hinton agar plates and incubated for 24 h at 37°C. The MBC was the lowest concentration which produced 99.9% reduction in CFU/ mL in comparison with the control. According to Berche *et al.*[19]; the antibacterial potential was deemed bactericidal or bacteriostatic depending on the ratio MBC/CMI. If MBC/MIC ≤ 4, the effect is bactericidal and when MBC/MIC > 4, it's bacteriostatic.

Antipyretic activity

The method described by Fadeyi *et al.*[20] was used to evaluate antipyretic activity of extracts of *Deverra scoparia* Coss. & Dur. Pyrexia was induced in rats by subcutaneous administration of brewer's yeast suspension aqueous brewer's yeast suspension (15% w/v in saline water, 10 mL/kg b.wt.) into the dorsum region of rats. The rectal temperature was taken by a rectal thermometer. After 19 h, animals that showed an increase of 0.5°C in rectal temperature were selected in this study. After, the animals were separated to eight groups of 6 rats. Group I, II and III were treated with infusion extract at 250, 500 and 1000 mg/kg b.w doses. respectively, Group IV, V and VI were received the ethereal extract at 250, 500 and 1000 mg/kg. Group VII served as pyrexia negative control which administered distilled water only. Group VIII as the reference drug was given paracetamol (500 mg/kg b.w.). Rectal temperature of all the rats was recorded at 1, 2, 3 and 4 h after orally administration of the treatments.

UPLC-MS analysis

The identification and quantification of the phenolic compounds presents in *Deverra scoparia* Coss. & Dur. extract was performed using Ultra performance liquid chromatography (UPLC) coupled with a photodiode array detector (PDA) in series with a mass spectrometry detector (MS). This analysis was carried out in chemistry laboratory of university of Lleida-Spain. The UPLC profil was obteined by Waters ACQUITY UPLC™ system (Waters, Milford, MA, USA) which consist of an ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager, all coupled to a photodiode array detector ACQUITY UPLC™ PDA. The separation of compounds was performed with column UPLC with characteristic 1.8 µm; 2.1 mm x 150 mm diameters (Waters, Manchester, UK). The mobile phase was a gradient of solvents consisting of solvent A, H_2O (0.1% v/v HAcO) and solvent B, ACN 100% (0.1% v/v HAcO). The flow rate was fixed at 0.50 mL/min. The linear gradient: 0-1.89 min, 1% B, (isocratic); 1.89 – 17.84 min, 30% B, (linear gradient); 17.84 – 21.39 min, 5% B, (linear gradient); 21.39 – 21.56 min, 1% B (linear gradient); 21.56 - 25 min, 1% B (isocratic). 20 µL of extract was injected in mode full loop; the temperature of the column was maintained at 45°C and at 10°C in the sample manager[15]. The MS analysis was performed on a Waters ACQUITY XEVO TQS tandem quadrupole mass spectrometer (Waters, UK) coupled with an electro-spray source (ESI) in positive and negative ion mode. The capillary voltage in positive and negative mode was of 3.0 kV and -2.5 kV respectively. The comparison of retention times between the UV and MS spectra of peaks with those of reference standards allowing the identification of phenolic compounds. Mass Lynx 4.1 software (Waters, USA) was used for data acquisition.

Statistical analysis

All results are expressed as mean ± Standard Deviation (SD). The one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. The values of $p<0.05$ were considered as significant.

Results

Antimicrobial activity

Antimicrobial activity of *Deverra scoparia* Coss. & Dur. extracts against the tested bacterial and fungal strains was assessed by disk diffusion method. The results of the diameters of inhibition zones are showed in the Table 1 and illustrated in Fig. 1 and 2.

Table 1 — Antimicrobial activity of *Deverra scoparia* Coss. & Dur. extracts against microorganisms tested

Microorganisms tested	Sampels Inhibition zone (mm)
Pseudomonas aeruginosa (G^-)	Infusion extract 46±0.04 Ethereal extract 43±0.01 Gentamicin 30±0.02
Escherichia coli (G^-)	Infusion extract 52±0.03 Ethereal extract 48±0.01 Gentamicine 36±0.05
Staphylococcus aureus (G^+)	Infusion extract 45±0.04 Ethereal extract 39±0.01 Vancomycin 33±0.02
Candida albicans	Infusion extract 46±0.01 Ethereal extract 32±0.01 Miconazole 31±0.02
Candida dubliniensis	Infusion extract 49±0.03 Ethereal extract 34±0.02 Miconazole 32±0.01

Data values are presented as (Mean ± SD), (n = 3)

The obtained results showed that the ethereal and infusion extracts possessed a net inhibition of the growth of the all microbial strains which are greater than the antibiotic tested. The infusion extract has an increased inhibition against microbial strains whose inhibition zones ranged from 45±0.004 mm and 52±0.003 mm. However, the ethereal extract presented a strong antimicrobial activity with inhibition zones of about 32±0.001 mm and 48±0.001 mm. It can be noted that all the microbial strains were susceptible against tested extracts.

The extracts were subjected to the determination of MIC and MBC values. These results are presented in Table 2.

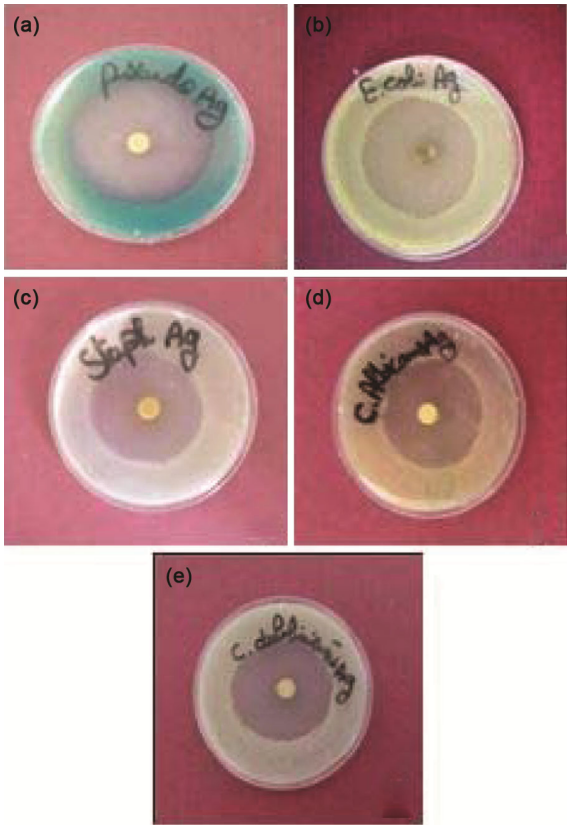

Fig. 1 — Diameters of inhibition zones of infusion extract *(A- Pseudomonas aeruginosa, B- Escherichia coli, C-Staphylococcus aureus, D- Candida albicans, E- Candida dubliniensis)*

The determination of the MIC and the MBC, allowed confirming the results of the antibacterial activity also to characterise the nature of the effect revealed by the extracts on each strain. These results relieved that the infusion and ethereal extracts showed the same MIC values (4 mg/mL) against *Escherichia coli* and *Staphylococcus aureus*. While, MIC values obtained against *Pseudomonas aeruginosa* were of 1 mg/mL and 4 mg/mL respectively. In addition, the MBC values of infusion extract were of 2 mg/mL, 8 mg/mL and 16 mg/mL against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus,* respectively. The ethereal extract presented the same MBC values against *Escherichia coli*

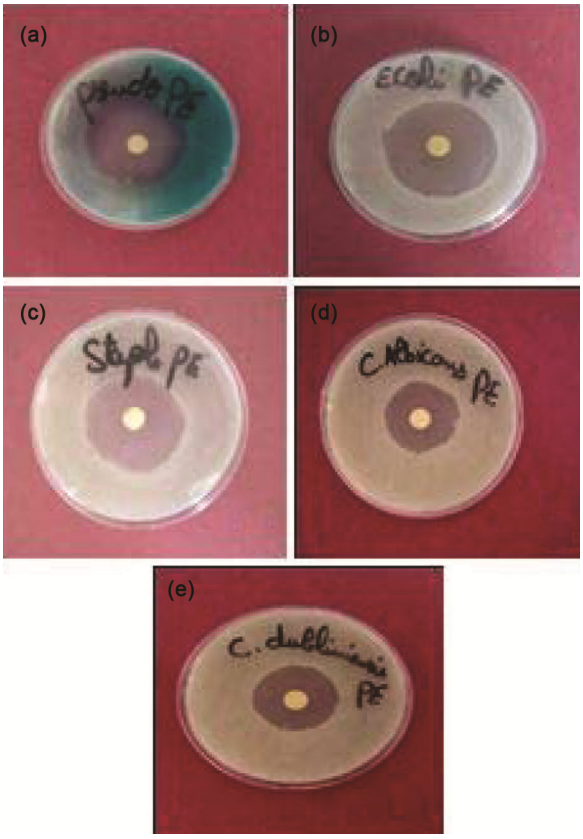

Fig. 2 — Diameters of inhibition zones of ethereal extract *(A- Pseudomonas aeruginosa, B- Escherichia coli, C-Staphylococcus aureus, D- Candida albicans, E- Candida dubliniensis).*

Table 2 — MIC, MBC and the MBC/MCI ratio on the antibacterial activity of the tested extracts

Microorganisms tested	Samples	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Pseudomonas aeruginosa (G^-)	Infusion extract	1	2	2
	Ethereal extract	2	16	8
Escherichia coli (G^-)	Infusion extract	4	8	2
	Ethereal extract	4	32	8
Staphylococcus aureus (G^+)	Infusion extract	4	16	4
	Ethereal extract	4	32	8

Data values are presented as (Mean ± SD), (n = 3)

and *Staphylococcus aureus* (32 mg/mL). For *Pseudomonas aeruginosa,* the MBC value was 16 mg/mL. From the obtained ratio MBC/MCI, it can be noted that the infusion extract showed bactericidal effect against all bacterial strains tested with values between 2 and 4. Contrarily, the ethereal extract exerts a bacteriostatic effect with a value equal to 8 for all strains.

Antipyretic activity

The results of the antipyretic effect of the infusion and ethereal extracts at doses of 250, 500 and 1000 mg/kg, compared with reference drug (paracetamol 500 mg/kg) are presented in Table 3 and Fig. 3.

The injection of yeast suspension induce the increase of the rectal temperature of rats compared to the initial rectal temperature. The rectal temperature of rats treated with paracetamol (500 mg/kg) decreased significantly ($p<0.05$) relative to the hyperthermic control group during the 4 h of the experiment. The administration of infusion extract significantly attenuated the temperature increase ($p<0.05$) compared to the control, this antipyretic effect is greater than that of paracetamol and of the ethereal extract. The decrease in temperature is dose-dependent manner; the antipyretic action started in the first hour until 4h of the order of 36.30; 36.14 and 36.10°C respectively for 250, 500 and 1000 mg/kg. In addition, the ethereal extract significantly ($p<0.05$) lowered the yeast induced elevated rectal temperature in a dose-dependent manner. The antipyretic effect is superior to paracetamol at the 500 and 1000 mg/kg of the order of 36.40, 36.30°C respectively at the 4th h.

UPLC-MS analysis

Individual phenolic compounds which could be responsible for the antimicrobial and antipyretic effects of *Deverra scoparia* Coss. & Dur. were identified and quantified by UPLC-MS as illustrated in Fig. 4.

The analysis in Fig. 4 shows the presence of caffeic acid and quercetin with a quantity of 16.4 and 1224.6 µg/g sample respectively.

Discussion

The uses of medicinal plants in the treatment of different health issues were passed down from generation to generation in history[22]. Plants are considered as sources of new drugs for human benefit due to their richness of bioactives substances that can be used to treat infectious diseases[23]. The present study evaluated the antimicrobial and antipyretic activities of infusion and ethereal extracts. The

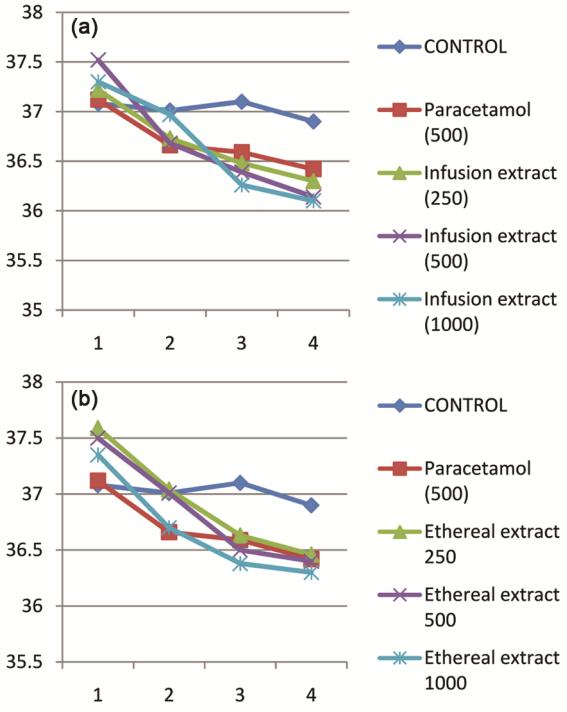

Fig. 3 — Effect of infusion extract (a) and ethereal extract (b) on yeast induced pyrexia

Table 3 — Antipyretic effect of *Deverra scoparia* Coss. & Dur. extracts and paracetamol in rats

Groups	Dose	Before treatment		After treatment		
		1 h	2 h	3 h	4 h	
Control		38.20±0.3	37.08±0.1	37.01±0.4	37.10±0.3	36.90±0.1
Paracetamol	500 mg/kg	38.10± 0.2	37.12± 0.2*	36.66± 0.3*	36.59± 0.1*	36.42± 0.1*
infusion extract	250 mg/kg	38.13± 0.4	37.22± 0.5*#	36.73± 0.3*#	36.48± 0.1*#	36.30± 0.2*#
	500 mg/kg	38.08± 0.3	37.52± 0.3*#	36.68± 0.1*#	36.39± 0.1*#	36.14± 0.3*#
	1000 mg/kg	38.16± 0.5	37.30± 0.4*#	36.97± 0.1*#	36.26± 0.3*#	36.10± 0.1*#
Ethereal extract	250 mg/kg	38.34± 0.4	37.59± 0.1*#	37.04± 0.2*	36.63± 0.1*#	36.46± 0.2*
	500 mg/kg	38.20± 0.2	37.52± 0.4*#	37.01± 0.3*#	36.50± 0.4*	36.40± 0.1*#
	1000 mg/kg	38.11± 0.1	37.35± 0.1*#	36.70± 0.2*#	36.38± 0.2*#	36.30± 0.3*#

*Data values are presented as (Mean ± SD) with n = 6. * $p<0.05$ significant compared to control; # $p<0.05$ significant compared to paracetamol (One-way ANOVA followed by Tukey's multiple comparison test).*

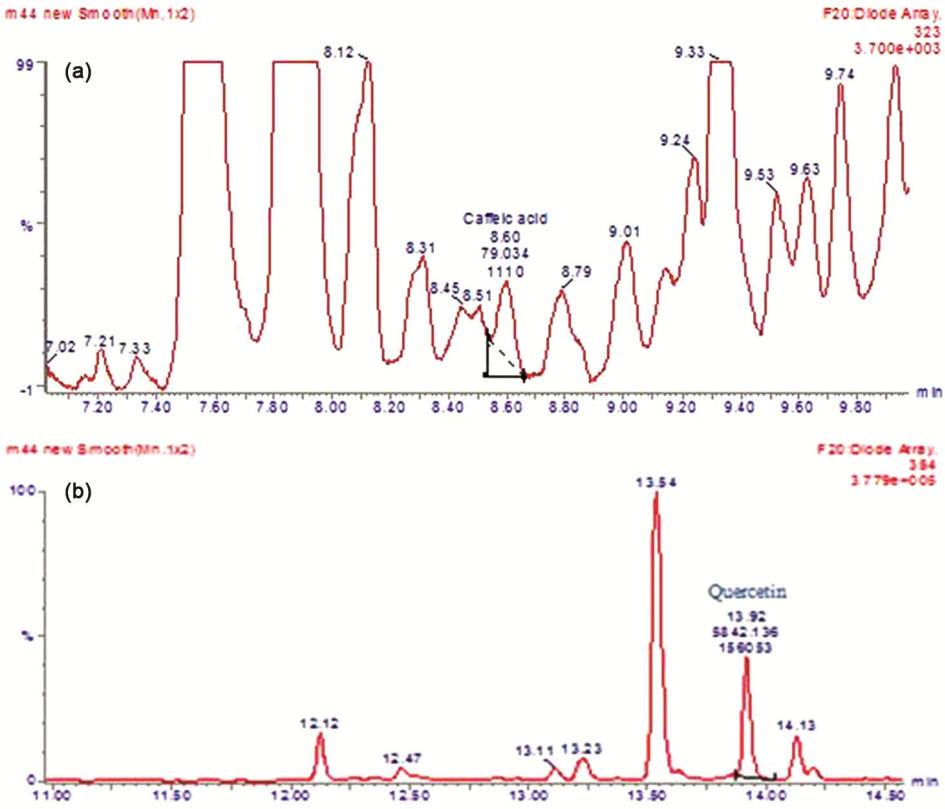

Fig. 4 — UPLC-MS chromatograms of caffeic acid (a) and quercetin (b)

antimicrobial activity was assessed using disk diffusion method which revealed that all the microorganisms tested were sensitive against the two extracts of *Deverra scoparia* Coss. & Dur. These results showed a large antimicrobial activity range by the extracts. Many studies showed that the antibacterial activity of medicinal plants could be due to the presence of phenolic compounds in their extracts. Their antibacterial mechanism is against the cytoplasmic membrane of bacterial cells, due to the hydroxyl groups[24]. The contact of hydrophobic phenolic groups and lipid bilayer could disrupt lipid–protein interaction rendering the bacterial cell membrane more permeable causing alterations in membrane structure. the destruction of bacterial membrane allows the entry of more hydroxyl groups[25]. Thereafter, the results of antipyretic activity of *Deverra scoparia* Coss. & Dur. extracts showed that the infusion extract at 250, 500 and 1000 mg/kg and ethereal extract with 500 and 1000 mg/kg possesses a significant greater antipyretic effect more than the reference drug used in this study. Pyrexia may be the result of inflammation, bacterial infections and other disease states[26]. The infectious or inflammation initiate the increased production of pro-inflammatory mediators cytokines such as (interleukin 1β, β, α and TNF-α) increasing the synthesis of prostaglandin E2 (PGE2) by activate arachidonic acid pathway near the hypothalamus area and the prostaglandins in turn elevate the body temperature[27,28]. Therefore, the possible mechanism of antipyretic action due to the prostaglandin synthesis inhibition[29]. These inhibition of prostaglandins due to the blocking of cyclo-oxygenase enzyme activity[30]. It can be suggested that the infusion and ethereal extracts contains bioactive compounds with antipyretic effect such as flavonoids which are known to target prostaglandins which are involved in the pyrexia[31]. Through an assay-guided by UPLC-MS analysis which indicated the presence of caffeic acid and quercetin with high quantity, these compounds were found to possess antimicrobial and antipyretic effects[32,33]. It has been reported that the antimicrobial action of caffeic acid was attributed to the depolerisation of cell membrane, may be associated with damage of cell membrane integrity[34]. Vaquero *et al.*[35] demonstrated that caffeic acid possesses stronger antibacterial activity than many phenolic

acid. The antibacterial mechanisms of action of quercetin have been attributed to inhibition of DNA gyrase of bacterial strains[36]. The antipyretic properties of caffeic acid and quercetin have been assessed by several authors; these bioactive compounds have the ability to reduce the elevated temperature by inhibition of prostaglandin E2 synthesis, also exhibit inhibition of arachidonic acid peroxidation[37,38]. These reports and the results obtained, suggest a relationship between the antimicrobial and antipyretic effects of the infusion and ethereal extracts and the presence of quercetin and caffeic acid in *Deverra scoparia* Coss. & Dur.

Conclusion

This experiment is a contribution to the pharmacological research in medicinal plants. It demonstrates that infusion and ethereal extracts of *Deverra scoparia* Coss. & Dur. possess an important antimicrobial and antipyretic affects which may be related to the presence of caffeic acid and quercetin in aerial parts of this plant. These results justify the use of this plant in phytotherapy of Saharan people.

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Conflict of Interest

All authors declare there is no conflict of interest.

Authors' Contributions

H L and O S wrote and revised the manuscript, C D revised the manuscript. All authors read and approved the final manuscript.

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