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Phytochemical Studies Antibacterial and Antioxidant Activities of Aerial Parts of *Ferula lutea* (Poir.) Maire.

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Abstract: *The focus of this paper was to assess the phytochemical composition, the antioxidant and antibacterial activities of the crude extracts of the aerial parts of Ferula lutea. Four new compounds were isolated from this genus, and their structures were primarily established by extensive spectroscopic analysis including 1D and 2D spectroscopic techniques. Total phenolics and flavonoids containing in ethyl acetate (AcOEt) and n-butanol (n-BuOH) extracts were quantified [199.1-184.02 mg gallic acid equivalent / g of dry weight], flavonoids [70.24-65.95 mg quercetin / g dry weight]. DDPH scavenging and phosphomolybdenum methods were used to evaluate in vitro antioxidant activity. The obtained results showed a significant antioxidant activity of the two aerial parts. Correlation analysis between the DPPH values and those for TAC indicates the possibility of the two models for evaluating antioxidants power from medicinal plants. Dichloromethane (CH_2Cl_2), AcOEt and n-BuOH extracts were screened against two gram-negative [Escherichia coli (E.coli) and Pseudo aeruginosa (P.aer)] and two gram-positive [Staphylococcus aureus (S.aur) and Bacillus (Bacil.)] bacteria using disc diffusion method. The results indicate a good inhibition of CH_2Cl_2 and EtAct extracts on the growth of (Bacil. and E.coli). Furthermore n-but extract showed a significant inhibitory effect only against E.coli.*

Keywords: *Ferula lutea*, phenolics, flavonoids, antioxidant, antibacterial.

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Introduction

Commonly known, natural products from medicinal plants represent one of the most important raw materials used for treating various human diseases because of the supreme availability of chemical diversity. With multiple biological activities, many plants contain antioxidant activity which attracts the attention of several research teams for its role in the fight against numerous illness and numerous studies have identified compound within herbal plants that are effective antibiotics [1]. The genus *Ferula* (Umbellifereae) has around 170 species [2], mostly growing in arid regions of temperate eurasia, in canary islands, in north Africa and in central Asia [3]. However, *Ferula* species are reputed in folk medicine for the treatment of skin infections [4], diabetes, prevent convulsion and hysteria [5] , rheumatism, arthritis, headache, dizziness and stomach pain [6]. In the framework of the valorization of the Algerian forest resources and to develop new products, we are interested in the present study by *Ferula lutea*, the selection of this plant was guided by its traditional use and also because only few studies were conducted on this plant.

Materials and Methods

Plant material

The aerial parts of *Ferula lutea* were collected during flowering stage from Setif region eastern of Algeria in May 2014 and were identified by Pr. H. Laouer from the department of biology and plant ecology (University of Setif 1, Algeria).A voucher specimen was deposited in the herbium of our laboratory (Chifa DZUMCAPPV00027).

Preparation of plant extracts.

1Kg of dried aerial parts of *F.lutea* was immersed in methanolic solution (MeOH, 70 %) at room temperature for 24 hours then extracted. The step was repeated for three times to extract the majority of the desired chemical components from the plant materials. The hydromethanolique extract was evaporated to dryness and the residue was dissolved in distilled water, filtered, and the filtrate was concentrated under vacuum with a rotary evaporator to give 255.81 g of methanolic extract. The residue was then extracted with CH_2Cl_2, EtOAc and *n*-BuOH successively in triplicate to give 6.8 g of CH_2Cl_2, 2.9 g of EtOAc and 25g of *n*-BuOH extracts.

Isolation of compounds

The CH_2Cl_2 extract (6.8g) was chromatographed over silica gel column chromatography (200 g, silica gel 60, Merck, 35-70 µm), starting the elution with a mixture of hexane / dichloromethane (from 100:0 to 0:100), followed by a mixture of dichloromethane/ethyl acetate (from 100:0 to 0:100), and finally with ethyl acetate / methanol (from 100:0 to 50:50). The obtained elutes were monitored using TLC plates viewed under UV light (254 and 365 nm) and by spraying 1% of vanillin solution, 2% of sulfuric acid/ethanol (H_2SO_4/EtOH) reagent followed by heating at 100 °C. The fractions [20-23] eluted with 70/30 hexane/dichloromethane (12 mg) showed one spot, which was purified with sephadex LH20 by analytical methanol to give 7 mg of white crystals: Methyl meristate (1) (Figure 1). Combined fractions [41-55] eluted with 40/60 hexane/dichloromethane (65mg) provide a mixture of a spot and chlorophyll. After purification of this fraction by acetone, 23 mg of white needles were obtained: Maltol (2). The fractions [107-109] eluted with 100% ethyl acetate showed one spot, which was purified with sephadex LH20 by analytical methanol to give (6 mg) white crystals: ß-sitostérol-3-O-ß-D-glucoside (3) (18 mg) and Spinasterol-3-O-ß-D-glucopyranoside (4) (5 mg) which was precipitated as an amorphous white powder from the AcOEt extract.

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Determination of total phenolic content

The total phenol content of the *F.lutea* extracts was measured spectrometrically according to the Folin Ciocalteu‘s method as described by Li et al [7]. dry extracts were solubilized with methanol to a final concentrations. A liquots of these samples (0.3ml) were mixed with 1.5 ml of the Folin-ciocalteu reagent (diluted 10 times with distilled water) and 1.2 ml of an aquous Sodium carbonate solution (7.5%) was added after 4 mn. Absorbance was read at 765 nm after 2 hours of incubation against a blank (solution with no extract added). Gallic acid was used to prepare a calibration curve and results are expressed in gallic equivalents (mg GA.E/g dry extraction).

Determination of total flavonoid content

The content in flavonoids was determined spectrophotometrically according to the $AlCl_3$ method developed by Djeridane [8]. Dry extracts were solubilized with methanol to a final concentration of (mg/ml). A liquots of these simples (1ml) were mixed with an equal volume of $AlCl_3$ solution (2% in methanol). The mixture was vigorously shaken and the absorbance was read at 430 nm after 10mn of incubation at ambient temperature. Quercetin was used to prepare a calibration curve and results are expressed as Quercetin equivalent (mg Q.E/g dry extract).

Antioxidant activity

Free Radical Scavenging DPPH Radical

The antioxidant activity for the *F. lutea* extracts were evaluated according to the method described by Yan [9]. Equal volumes of the methanolic solution of 1,1-diphenyl 1.2-picrylhydrazyl (DPPH, 0.2 mM) and of the extracts (at the various concentrations indicated) were mixed and kept at room temperature for 30 mn. The absorbance was denoted at 517 nm, beside a methanol solvent as control solution.

$$\text{The scavenging} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

where: A control is the absorbance at 517nm of DPPH solution without addition of the extracts. A sample is the absorbance at 517nm of extracts. Ascorbic acid was used as a reference standard. IC_{50} values (concentration of extract necessary to reduce by 50 % of the initial quantity of DPPH).

Total antioxidant capacity (TAC).

Total antioxidant capacity (TAC) of *F. lutea* extracts were spectrophotometrically determined by the phosphomolybdenum assay using the method described by Pieto [10]**.** Briefly, 0.3 mL of methanolic extracts solutions (2mg/mL) was mixed with 3 mL of phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in capped tubes. Incubation was then carried out for 90 min in water bath at 95°C. After cooling to room temperature; the absorbance of the solutions was measured using UV-visible spectrophotometer at 695 nm against a control solution (0.3 mL methanol without extract). TAC results were expressed as ascorbic acid equivalents (mg A.A.E/g of dry sample).

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Antimicrobial activity

Microbial strains.

All clinical strains bacteria [*Staphylococcus.aureus, Bacillus, Escherichia.coli, Pseudomonas. aeruginosa*], were obtained from laboratory of bacteriology at Constantine University hospital (C.H.U).

Inhibitory effect and data processing.

The CH_2Cl_2, EtOAc and *n*-BuOH extracts of *F.lutea were* investigated for their antibacterial activity using disk diffusion method [11], where the bacterial strains were first cultured on Mueller-Hinton agar for 24 hours at 37 °C. A sterilized filter disk (6 mm diameter from Whatman paper N°3) was placed on the infusion agar seeded with bacteria and impregnated with 40 µl of the extract suspended in ethanol 60%, for all concentration (2mg/ml, 1mg/ml, 0.5 mg/ml, 0.25 mg/ml). After that Petri dish were left at 4 °C for 1 h, and then incubated at 37 °C for 24 hours. The diameters of inhibition zones were measured in millimeters and all tests were performed in triplicate. The antibacterial potential extracts were assessed in terms of inhibition zone of bacterial growth. The results of the antibacterial activity were expressed as means ± SD and presented in tables 1-3. The control treatment (ethanol 60%) had no inhibitory effect on any of the tested microorganisms.

Results and Discussion

Identification of compounds

Compound(1): $C_{15}H_{30}O_2$, white crystals, M= 242.40g/mol. ^{1}H-NMR (600 MHz, $CDCl_3$, δ/ppm, J/Hz) 0.87(*t*, 3H, *J*=7.2, CH-3), 1.25(20H, *s*, H-4, H-13), 1.63(2H, *m*, H-3), 2.35(2H, *t*, *J*=7.2, H-2), 3.67(3H, *m*, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$, δ/ppm): (C-1) 179.28, (C-2) 34.10, (C-3) 24.94, (C-4 to C-11) 29.1 4-29.66, (C-12) 31.9, (C-13) 22.67, (C-14) 14.09, (C-15) 51.39. It was identified as Methyl meristate [12]**.**

Compound (2): $C_6H_6O_3$, colorless needles, M= 126g/mol. ^{1}H-NMR (250 MHz, $CDCl_3$, δ/ppm, J/Hz): 2.39(3H, *S*, CH-*3*), 5.32(1H, *s*, OH), 6.46(1H, *d*, *J*= 5.5, H-5), 7.74(*1H*, *d* *J*=5.5, H-6) . ^{13}C-NMR (75 MHz, $CDCl_3$, δ/ppm): (C-2) 154.30, (C-3) 149.53, (C-4) 173.23, (C-5) 113.27, (C-6) 143.33, (C-7) 14.44. It was identified as Maltol [13].

Compound (3): $C_{35}H_{60}O_6$, white amorphous powder, M= 576.859g/mol. ^{1}H-NMR (600 MHz, DMSO-d6,, δ/ppm, J/Hz): 0.65(3H, *s*, H-18), 0.79(9H, *s*, H-26, H-27 and H-29), 0.90(1H, *d*, *j*=5.4, H-21), 0.95(3H, s, H-19), 2.00(1H, *m*, H-20), 3.02(1H, *m*, H'-4), 3.07(1H, *m*, H'-3), 3.12(1H, *m*, H'-5), 3.39(1H, *m*, H'-6a), 3.58(1H, *m*, H-3), 3.65(1H, *m*, H'-6b), 4.22(1H, *d*, *j*=7.2, H'-1), , 5.33(1H, *s*, H-6).^{13}C-NMR, (150 MHz,DMSO-d6): (C-1) 37.28, (C-2) 29.71, (C-3) 77.36, (C-4) 40.48, (C-5) 140.89, (C-6) 121.65, (C-7) 31.87, (C-8) 31.82, (C-9) 50.05, (C-10) 36.66, (C-11) 21.04, (C-12) 38.75, (C-13) 42.30, (C-14) 56.62, (C-15) 24.31, (C-16) 28.24, (C-17) 55.87, (C-18) 12.12, (C-19) 19.55, (C-20) 35.93, (C-21) 19.07, (C-22) 33.79, (C-23) 25.88, (C-24) 45.58, (C-25) 29.15, (C-26) 19.39, (C-27) 20.17, (C-28) 23.05, (C-29) 12.24, (C-1') 101.21, (C-2') 73.91, (C-3') 77.20, (C-4') 70.55, (C-5') 77.18, (C-6') 61.54. It was identified as ß-sitosterol -3-*O-ß*-D-glucoside [14].

Compound (4): $C_{35}H_{58}O_6$, white amorphous powder, M= 574.423g/mol. ^{1}H-NMR (600 MHz DMSO-d6, δ/ppm, J/Hz): 0.50(3H, *s*,H-18), 0.72(3H, *s*, H-19), 0.76(6H, *s*, H-26 and H-29), 0.81(3H, *d*, *J*=6, H_{27}), 0.98(3H, *d*, *J*=6.6, H-21), 1.93(1H, *d*, *j*=11.4, H-5), 3.39(1H, *m*, H-'6a),

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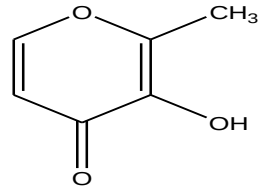
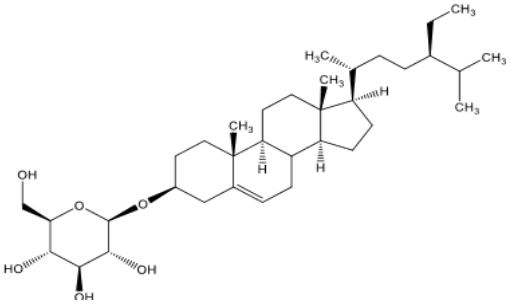
3.53(1H, *m*, H-3), 3.62(1H, *m*, H'-6b), 4.2(1H, *d*, *J*=7.8, H-1'), 5.02(1H, *dd*, *J*=9, 15, H-23), 5.10(1H, *s*, H-7), 5.14(1H, *dd*, *J*=9, 15, H-22), ^{13}C-NMR (DMSO-d6, 150 MHz, δ/ppm): (C-1) 37.00, (C-2) 29.54, (C-3) 76.75, (C-4) 34.40, (C-5) 40.52, (C-6) 29.67, (C-7) 117.66, (C-8) 139.47, (C-9) 49.11, (C-10) 34.40, (C-11) 21.70, (C-12) 39.70, (C-13) 43.28, (C-14) 54.93, (C-15) 22.99, (C-16) 28.57, (C-17) 55.66, (C-18) 12.32, (C-19) 13.24, (C-20) 40.74, (C-21) 21.45, (C-22) 138.37, (C-23) 129.43, (C-24) 51.05, (C-25) 31.77, (C-26) 19.30, (C-27) 21.42, (C-28) 25.33, (C-29) 12.57, (C-1') 101.30, (C-2') 73.94, (C-3') 77.20, (C-4') 70.55, (C-5') 77.15, (C-6') 61.57. It was identified as Spinasterol 3-O-ß-D- glucopyranoside [15].

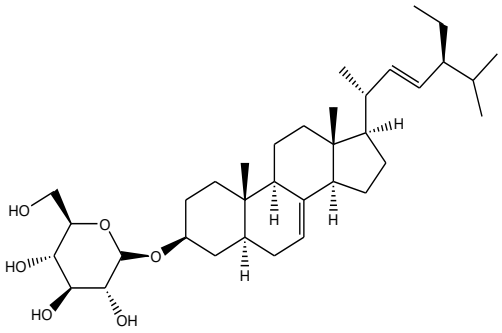
Methyl meristate



Maltol



ß-sitosterol -3-*O-ß*-D-glucoside



Spinasterol 3-O-ß-D- glucopyranoside

Fig.1: Structures of compounds isolated from aerial parts of *Ferula lutea*.

Total phenolic and flavonoid contents.

Total phenolic and flavonoid contents of ethyl acetate and butanolic extracts of aerial parts of *Ferula lutea* are illustrated in table 1.

Table 1. Total phenolic and flavonoid contents of the AcOEt and BuOH extracts of aerial parts of *Ferula lutea.*

Plant	Extract	Phenol contents [(a)]	Flavonoid contents [(b)]
Ferula lutea	AcOEt	199.10 ± 2.05	70.24 ± 0.87
	BuOH	184.02 ± 2.84	65.95 ± 1.25

a: expressed as mg Gallic acid /g of extract, b: expressed as mg quercetin /g of extract.

The results obtained in this study (table 1) showed a significant level of phenolic compounds in the two studied extracts, where we found remarkable values for both extracts; 199.10 ±2.05 and 184.02± 2.84 mg GAE/g of extracts (successively AcOEt extract and ButOH extract). The flavonoid content of the AcOEt extract was the highest with 70.24± 0.87 mg QE/g extract while the n- butanol extract had 65.95±1.25 mg QE/g extract. the phenolics and flavonoids content of aerial parts of *Ferula lutea* has not been reported in the literature, but

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we find the study of Manssour Zenati (*F.lutea*, flowers) showed that the total phenolics were 52.3± 0.4 and 40.7± 0.2 mg GAE/g of dry weight and the total flavonoids were 14.7 and 12.4± 0.1 mg QE/ g of dry weight (successively AcOEt extract and ButOH extract) [16].

Free Radical Scavenging DPPH Radical

The antioxidant activity of the extracts was investigated using DPPH and TAC is presented in **tables II** and **III**. By the DPPH test, the best result was obtained with *n*-BuOH extract IC_{50} = 0.12 mg/ml) followed by the ethyl acetate extract IC_{50} = 0.13mg/ml. the two extracts have good radical scavenging activity in comparison with positive control Ascorbic acid (Vit C, IC_{50} = 0.04 mg/ml). Total antioxidant capacity of extracts was determined as 181.45±0.25 for EtOAc extract and 175.78±2.51 for *n*-BuOH extract. In addition, the antioxidant capacities obtained from the DPPH scavenging assay were in good accordance with those obtained from TAC assay, which implies that the antioxidants in this plant were able to scavenge free radicals and reduce oxidants where we find n-butanol extract possessed high radical scavenging activity, but ethyl acetate extract possessed high oxidant reducing activity. Presence of this phytochemicals in aerial parts of *F. lutea* provides some scientific evidence for the traditional usage of this plant.

The antioxidant activity (DPPH, TAC) aerial parts of *F. lutea* extracts, has not been cited in the literature before**[**16]. Only, *F.lutea* flowers was evaluated for antioxidant activity with DPPH assay giving an IC_{50} = 12.8 ± 1.3 µg/ml and IC_{50} = 26.0 ± 1.8 µg/ml and with ABTS assay giving an IC_{50} = 184.0±7 µg/ml and IC_{50} = 300 ± 5 µg/ml successively for AcOEt and n-BuOH extracts.

Table 2. DPPH scavenging activities of EtOAc and BuOH extracts of aerial parts of *Ferula lutea.* Ascorbic Acid were used as positive control

	Linear Equation	IC_{50}	APR
Ascorbic acid	Y=1506x – 10.20 (R^2=0.995)	0.04	25
EtOAc	Y=325x +7.090 (R^2=0.972)	0.13	7.69
BuOH	Y=347.9x +7.506 (R^2=0.974)	0.12	8.33

All analyses are the mean of triplicate measurement ± standard deviation.

Table 3. Total activity capacity of the AcOEt and ButOH extracts of aerial parts of *Ferula lutea.*

Extract	EtOAc	BuOH
Total antioxidant capacity	181.45 ± 0.25	175.78 ± 2.51

All analyses are the mean of triplicate measurement ± standard deviation.

Antibacterial activity

The diffusion test was applied to 4 Gram positive and Gram negative microorganism. The control treatment (ethanol 60 %) had no inhibitory effect on any of the tested microorganism and the results of these tests are illustrated in tables I, V and VI.

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Table 4. Antibacterial activity of the extract CH_2Cl_2.

	CH_2Cl_2				Ethanol 60%
Microorganismes	0.25mg/ml	0.5mg/ml	1mg/ml	2mg/ml	-
S.aureus	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	-
Bacillus	17.00 ± 1.7	17.00 ± 1.7	17.00 ± 1.7	20.00 ± 0.9	-
E.coli	14.33 ± 1.2	14.33 ± 1.2	14.33 ± 1.2	15.00 ± 1.0	-
p.aeruginosa	07.33 ± 1.2	07.33 ± 1.2	07.33 ± 1.2	06.67 ± 1.2	-

Table 5. Antibacterial activity of the extract EtOAc.

	EtOAc				Ethanol 60%
Microorganismes	0.25mg/ml	0.5mg/ml	1mg/ml	2mg/ml	-
S.aureus	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	-
Bacillus	15.00 ± 1.7	15.00 ± 1.0	16.00 ± 0.0	17.33 ± 1.2	-
E.coli	12.00 ± 1.7	14.33 ± 1.2	16.66 ± 1.2	17.00 ± 1.2	-
p.aeruginosa	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	06.0 ± 0.0	-

Table 6. Antibacterial activity of the extract *n*-BuOH

	n-butanol				**Ethanol 60%**
Microorganismes	0.25mg/ml	0.5mg/ml	1mg/ml	2mg/ml	-
S.aureus	06.00 ± 0.0	06.00 ± 0.0	06.00 ± 0.0	06.00 ± 0.0	-
Bacillus	06.00 ± 0.0	06.00 ± 0.0	06.00 ± 0.0	06.00 ± 0.0	-
E. coli	16.66 ± 0.6	17.66 ± 0.6	18.70 ± 1.2	18.80 ± 1.0	-
p.aeruginosa	07.00 ± 0	07.66 ± 1.2	07.00 ± 0.0	06.00 ± 0.0	-

The antibacterial activity of *F.lutea* aerial parts extracts against microorganism was examined in the present study. The microorganisms *S. aureus*, which is already known to be multi-resistant to drugs and *P. aeruginosa*, which is also resistant to different antibiotics, were also resistant to the plant extracts tested. On the other hand, *n*-butanol extract has no activity against *Bacillus*. The n-BuOH, EtOAc and CH_2Cl_2 extracts showed a good activity against *E. coli* with higher concentration of 2mg/ml and presented an inhibition zone 18.80 ± 1.0, 17.00 ±1.2 and 15.00 ± 1.0 mm, respectively (tables I, V and VI). CH_2Cl_2 extract exhibited the best activity against *Bacillus* and showed an inhibition zone 20.00 ± 0.9 mm with high concentration of 2mg/ml, the EtOAc extract presented also a good activity with 17.33 ±1.2 mm at the last concentration (table V). Among the tested extracts, the CH_2Cl_2 and EtOAc were more effective than n-BuOH extract.

The n-butanol extract exhibited more interesting antimicrobial activities than the ethyl acetate extract (*Ferula lutea*, flowers) **[**16]. .

Conclusion

The results obtained in this work find four compounds were isolated the first time from the extracts obtained from the aerial parts of *Ferula lutea*, the structures of the isolated compounds were identified by extensive spectroscopic analysis including 1D and 2D spectroscopic techniques. The compounds are: Methyl meristate (1), Maltol (2), ß-sitosterol-3-O-ß-D-glucoside (3), spinasterol-3-O-ß-D-glucopyranoside (4).

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The present study demonstrated that total phenolic and total flavonoid content varied between the tested extracts. In addition, the DPPH radical scavenging activity and TAC assay were found to be significantly correlated with the amount of TPC and TFC. In this work, it is confirmed that the plant extracts tested not only as antioxidant but also as antibacterial, when we find the antibacterial activity of the dichloromethane and acetate extracts was especially very strong against *Bacillus* and butanol extract was very strong against *E.coli.*

The present results therefore offer a scientific basis for traditional use of *Ferula lutea.*

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