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Original Research Article

HPLC-DAD Analysis and Antioxidant Potential of *Ferula assa foetida* Resin Ethanol Extract

Hichem Beladjal*, Djilali Bouhadi, Hamza Belkhodja

Laboratory of Bioconversion, Microbiology Engineering and Health Safety, University of Mustapha Stambouli, Mascara, 29000, Algeria

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ABSTRACT

Ferula assa foetida, commonly consumed as a healthy beverage, has been demonstrated to have various biological activities, including antioxidant activity. The present study aims to investigate the chemical composition and the antioxidant effect of *F. assa foetida* resin ethanol extract. Qualitative phytochemical screening of the extract was done according to standard procedure. The chemical constituents of *F. assa foetida* resin ethanol extract was identified using high performance liquid chromatography-diode array detector-electrospray ionization-mass spectrometry (HPLC-DAD-ESI-MS) analysis. The antioxidant potential was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and total antioxidant capacity assays. Qualitative phytochemical analysis revealed the presence of various bioactive phytochemicals including steroids, saponins, flavonoids, phenols, coumarins, and cardiac glycosides. Eleven compounds were identified from the HPLC analysis of the ethanol extract of *F. assa foetida* resin. These include vanillic acid, p-coumaric acid, ferulic acid, 3,4-dihydroxybenzoic acid, chlorogenic acid, sinapic acid, caffeic acid derivative, chrysin, rosmarinic acid, umbelliprenin and galbanic acid. The extract exhibited high DPPH free radical scavenging activity with a percentage inhibition of 96.5% against 98.3% for ascorbic acid. In addition, the ethanol extract of *F. assa foetida* displayed a moderate total antioxidant capacity with a value of 3.31 mg AAE/g. On the basis of the findings from this study, *Ferula assa foetida* resin extract could therefore be considered as an alternative source of antioxidant agent that can prevent against oxidant stress-induced damage.

Keywords: Antioxidant, Extraction, *Ferula assa foetida* resin, Oxidative stress, Polyphenols.

Introduction

Secondary metabolites, which were once viewed as useless plant material, are now regarded as essential components with immense medicinal potential. As a result of their widespread use in drug development today, natural secondary metabolites found in plants are thought to have pharmacological qualities that humans have employed since the dawn of time.[1] Natural substances with therapeutic properties have been used since the dawn of civilization. The primary sources of medications continue to be those derived from animals, plants, and minerals.[2] Many biochemical processes in the body create reactive oxygen species, which are capable of causing harm to critical biomolecules. They result in disease states if biological components do not efficiently scavenge them.[3] Antioxidant chemicals that scavenge free radicals and cleanse the body can prevent the damaging effects of free radicals.[4]

Natural compounds have shown their importance as historically recognized medicinal agents, and researchers have sought alternatives to synthetic antioxidants.[5] In reality, a significant fraction of the most widely used pharmaceuticals were first made from natural ingredients.[6] Herbs are a significant source of new bioactive products for producing pharmaceuticals.[7]

*Corresponding author. E mail: hichem.beladjal@univ-mascara.dz
Tel: 00213656656359

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The ability of these herbal remedies to prevent or reduce damage by free radicals to macromolecules, including lipids, proteins, and DNA, has been linked to the effectiveness of these substances.[8]

Ferula assa foetida (asafoetida) is used as an oleo gum resin obtained by stem and root incision.[9] Recent investigations in pharmacology and biology have confirmed the pharmacological effects of this gum resin, including antioxidant, anticonvulsant, antispasmodic, antidiabetic, antileishmanial, hypotensive, and antinociceptive properties.[10,11] For this purpose, the objective of the present study was to investigate the chemical composition followed by an evaluation of the antioxidant potential of *F. assa foetida* resin ethanol extract.

Materials and Methods

Plant materials

Ferula assa foetida resin was purchased from a grocer in the Mascara region (Algeria) (35° 24′ 00″ north, 0° 08′ 00″ east) in April 2022. The plant material was identified by a botanist - Prof. Righi Kada, Department of Agronomy, University of Mustapha Stambouli, Mascara. Herbarium specimen with the voucher number 2365 deposited. The resin was ground into powder by a mechanical grinder and stored in sealed glass bottles until used.

Extraction of plant materials

F. assa foetida resin (3 g) was extracted by decoction with 60 mL of ethanol. The extract was filtered using Whatman Filter paper No. 1. Thereafter, the filtrate was concentrated using a rotary evaporator (Hei-VAP Ultimate Control, Germany) at reduced pressure. The percentage yield of the resulting extracts was calculated, and then the extract was stored in the refrigerator (Fisherbrand™ Isotemp Value, Germany) until used.

Qualitative phytochemical analysis
Phytochemical composition of the extract of *F. assa foetida* was determined using different conventional techniques based on colour change and precipitation.[12,13]

Determination of total phenolic content
Determination of total phenolic content was performed using the Folin-Ciocalteu reagent.[14] A mixture of 1 mL of sodium carbonate and 0.25 mL of extract or standard (gallic acid) at different dilutions were added to 1.25 mL of Folin-Ciocalteu. The mixture was allowed to stand at room temperature for 90 min. The absorbance was measured at 765 nm using a spectrophotometer (UV-1280 UV-Visible, Germany). The total phenolic content of the extract was calculated from the regression equation obtained from the gallic acid calibration curve. The result was expressed as mg gallic acid equivalent/g (mg GAE/g).

Determination of total flavonoid content
The total flavonoid content of the extract was determined using the Aluminium chloride colorimetric method.[15] To 0.3 mL of 5% sodium nitrite ($NaNO_2$), 1 mL of the extract (or quercetin solution in different dilutions) was added. After 5 minutes, 0.3 mL of aluminum trichloride ($AlCl_3$) (10% in methanol) and 2 mL of 4% sodium hydroxide (NaOH) were added, the volume was made up to 10 mL with distilled water. The absorbance of the resulting mixture was measured at 510 nm using a spectrophotometer. The total flavonoid content was calculated from the regression equation of quercetin calibration curve and the values were expressed as mg quercetin equivalent/g.

Determination of total tannin content
The total tannin content of the extract was determined using vanillin-hydrochloric acid technique.[16] Briefly, 3 mL of 4% vanillin and 1.5 mL of hydrochloric acid was mixed and then added to 0.5 mL of the extract or tannic acid at different concentrations. The mixture was allowed to stand at room temperature for 15 min. Then, the absorbance was measured at 500 nm. Using the equation derived from the tannic acid calibration curve, the total amount of condensed tannins was determined as mg tannic acid equivalent/g (mg TAE/g).

HPLC-DADESI-MS analysis
A Shimadzu system (prominence I. LC-2030C 3D) outfitted with a surveyor UVVIS diode array detection (DAD), an LCQ advantage max ion trap mass spectrometer, and a linked electrospray ionization (ESI) source was used to identify the polyphenols and flavonoids in *F. assa foetida* extract. The Ascentis express C18 column (15 cm x 4.6 mm) ID packed with 2.7 m partly porous particles used as the separation platform (Supelco, Bellefonte, PA, USA). Water/acetic acid (0.075%; Solvent A) and methanol/acetic acid (0.075%; Solvent B) made up the binary mobile phase (solvent B). The gradient was 2% B for the first five minutes, 2%-100% B for 5-80 minutes, and 100% B for 80-85 minutes. The injection volume was 5 µL, and the flow rate was 0.8 mL/min. The chromatograms were detected at 280 nm, and the Photometric Diode Array wavelength range was 190-400 nm. The following settings were used for MS acquisition utilizing an ESI interface: Interval: 0.5 seconds; scan speed: 1500 amu/s; flow rate of nebulizing gas (N2): 1.5 L/min; interface temperature: 350°C, heat block temperature: 300°C; desolvation line temperature: 300°C; DL voltage: 34 V; interface voltage: 4.5 kV; Q array DC voltage: 1.0 V; Q array radio frequency voltage: 60 V. Mass spectral range: 100-800 m/z. The data were presented as micrograms per gram dried extract %RSD and were calculated from the average of three assays.

Determination of DPPH radical scavenging activity
The antiradical potential of the extract was performed using the 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging method as previously described by Miki *et al*. (2021).[17] Fresh DPPH radical solution was prepared by dissolving 2 mg of DPPH in 50 mL of methanol. To 1.95 mL of the DPPH solution was added 200 µL of the extract or standard solutions (ascorbic acid) at various concentrations. The mixture was incubated at room temperature in the dark for 30 min. Thereafter, the absorbance of the mixture was measured at 517 nm against a blank solution which contained only the DPPH. The antioxidant activity of the extract or standard was assessed on the basis of the percentage inhibition of DPPH radical as calculated from the equation below.

$IP = (Abs_{Control} - Abs_{Extract})/Abs_{Control} \times 100$
Where;
IP = Inhibition percentage.
Abs Control = Absorbance of the negative control.
Abs Extract = Absorbance of the sample.
The concentration that corresponds to 50% inhibition (IC_{50}) was calculated by monitoring the variation of antiradical activity with the extract concentration. A low IC_{50} value indicates a high antioxidant activity.

Determination of total antioxidant capacity (TAC)
The total antioxidant capacity of the extract was evaluated by the phosphomolybdate assay as described by Purwoko *et al*. (2022).[18] The phosphomolybdate reagent solution (3 mL) comprising 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to 0.3 mL of the extract. The mixture was incubated for 90 minutes at 95°C in an incubator (CLN32, Poland). After cooling, the absorbance of the solution was measured at 695 nm against a reagent blank. Ascorbic acid was used as the reference standard, and the total antioxidant capacity was expressed as milligram ascorbic acid equivalents per gram of dry matter (mg AAE/g).
Statistical analysis
Statistical analysis was performed using SPSS version 20. One-way analysis of variance (ANOVA) was used to detect the significant difference between the extract and standard in the antioxidant studies. A probability value $P \leq 0.05$ is considered to represent a statistically significance difference.

Results and Discussion

Phytochemical constituents
The qualitative phytochemical analysis of the extract was aimed at highlighting the secondary metabolites in the resin. The detection of these chemical compounds was based on solubility, precipitation, turbidity, and colour reactions, where the intensity of the colour is proportional to the quantity of the substance sought. The result of the preliminary phytochemical screening of the resin extract of *Ferula assa foetida* is presented in Table 1. The result revealed the presence of various bioactive compounds with the remarkable presence of steroids, saponins, flavonoids, phenols, coumarins, and cardiac glycosides. Tannins were present in little quantity, while terpenoids, anthraquinones, and alkaloids were absent.

Table 1: Phytochemical constituents of *Ferula assa foetida* Resin Ethanol Extract

Phytochemical	Inference
Steroids	+
Terpenoids	-
Tannins	+
Flavonoids	+
Alkaloids	-
Saponins	+
Phenols	+
Anthraquinones	-
Glucoside cardiotonic	+
Coumarins	+

(+) indicates presence of compound, (-) indicates absence of compound

In general, the extraction of these phytochemicals is influenced by the extraction methods chosen. Other factors, such as pH, temperature, and the ratio of the quantity of matter to the volume of the solvent, also play an essential role in the variation in the quantity of the phytochemicals.[19,20] All of the phytochemical groups identified have remarkable pharmacological properties.[21,22] The therapeutic potential of a medicinal plant has been attributed to the action of the phytochemical constituents.[23]

The concentrations of polyphenols and flavonoids in *Ferula-assa-foetida* resin extract were determined from the regression equations obtained from the calibration curves ($y = 0.005x + 0.0137$, $R^2 = 0.9982$) and ($y = 0.0032x + 0.1137$, $R^2 = 0.9487$) plotted using the reference standards gallic acid and quercetin, respectively. The concentration was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract) for polyphenols and in milligrams of quercetin equivalent per gram of extract (mg QE/g extract) for flavonoids. Condensed tannin contents were expressed as milligrams of tannic acid equivalent per gram of extract (mg TAE/g extract) obtained from the regression equation of tannic acid calibration curve ($y = 0.0021x + 0.0133$) with a coefficient of determination ($R^2 = 0.9637$). The quantitative phytochemical analysis result revealed that the ethanol extract of *Ferula assa foetida* has substantial contents of total polyphenols, flavonoids, and tannins with values of 125 mg GAE/g, 23.7 mg QE/g, and 0.15 mg TAE/g for total polyphenols, total flavonoids, and total tannins, respectively.

According to Yazdanipour *et al*. (2021),[24] the total phenolic content of the ethanol extract of *Ferula assa-foetida* oleo-gum-resin was 29.5 mg of GAE/g, and the total flavonoid content was 6.1 mg QE/g. The results of Niazmand *et al*. (2021),[25] revealed that flavonoid content in leaf extract of *Ferula assa-foetida* was 16.71 mg QE/g, while in the gum extract, it was only 0.11 mg QE/g. These results were in agreement with those of Agour *et al*. (2021),[26] who reported that the flavonoid content of *F. assa foetida* leaf hydroalcoholic extract and *F. assa foetida* leaf essential oil were 12.53 mg/100 g and 0.015 mg/100 g, respectively. Another study that measured the total phenolic content of Ferula revealed that it contained 36.4 mg of phenolic components per gram of the extract.[14] Phenolic and flavonoid compounds have considerable health-improving effects and are essential pharmaceutical ingredients.[27]

Compounds identified from HPLC analysis

A total of 11 compounds were identified in the ethanol extract of of *Ferula assa-foetida* resin (Figure 1). These include; Vanillic acid, p-Coumaric acid, Ferulic acid, 3,4-Dihydroxybenzoic acid, Chlorogenic acid, Sinapic acid, Caffeic acid derivatives, Chrysin, Rosmarinic acid, Umbelliprenin, and Galbanic acid. In particular, Ferulic acid was present in significantly higher amount (10.42%) than the other compounds, this was followed by Rosmarinic acid (4.69%) (Table 2). These results were in agreement with those of Niazmand *et al*. (2021),[25] who revealed that the HPLC analysis of *F. assa foetida* leaf ethanol extract identified 7 phenolic compounds, including vanillic acid, ferulic acid, coumaric acid, umbelliprenin, karatavicinol, kamolonol, and galbanic acid. According to the study of Zengin *et al*. (2018),[28] 42 compounds were identified from the alcohol extract of *F. assa foetida.* According to Yazdanipour *et al*. (2021),[24] 7 compounds including ferulic acid (21.55%), gummosin (3.39%), farnesiferol (6.57%), Galbanic acid (7.33%), samarcandin (11.35%), asafoetida (3.41%) and umbelliprenin (6.66%) were the most abundant in the ethanol extract of *Ferula assa-foetida* oleo-gum-resin. Additionally, the work of Sgarbossa *et al*. (2015),[29] illustrated the neuroprotective potential of ferulic acid as a therapeutic agent in Alzheimer's disease. They demonstrated the ability of ferulic acid to inhibit amyloid beta aggregations both *in vitro* and *in vivo* and modulate oxidative stress-induced apoptotic programmed cell death induced by oxidative stress. Membrane characteristics, including permeability, charge, and even physicochemical properties, were irreversibly modified by ferulic acid due to changes in hydrophobicity, generation of localized ruptures or pores, and a reduction in negative surface charge in the cell membranes.[30]

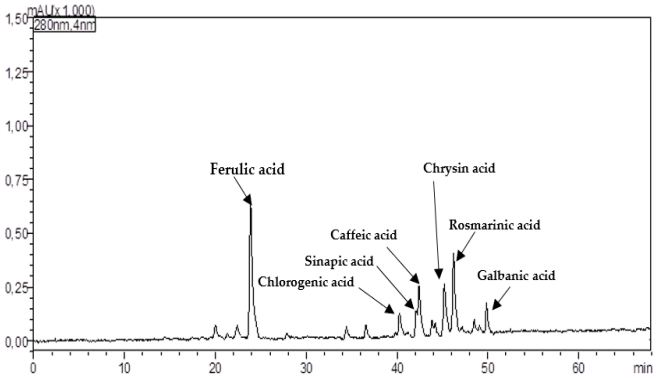

Figure 1: Chromatogram of HPLC analysis of *Ferula assa foetida* resin ethanol extract

Table 2: Compounds identified from the HPLC analysis of *Ferula assa foetida* resin ethanol extract

Peak	RT	Compound	Area	% Area
1	20.006	Vanillic acid	1987423	0.929
2	22.407	*p*-coumaric acid	2402353	1.122
3	23.877	Ferulic acid	22310044	10.424
4	34.427	3,4-dihydroxybenzoic acid	1893301	0.885
5	36.555	*p*-coumaric acid	2084492	0.974
6	40.260	Chlorogenic acid	3469967	1.621
7	42.118	Sinapic Acid	2291224	1.071
8	42.426	caffeic acid derivatives	6432597	3.006
9	45.219	Chrysin	7351688	3.435
10	46.217	Rosmarinic acid	10045160	4.694
11	48,507	Umbelliprenin	1921072	0.898
12	49.845	Galbanic acid	3929214	1.836

Ferulic acid antioxidative potential was attributed to the reaction of the antioxidant molecule with a radical to form a stable radical, which impedes the initiation of complex reaction cascades and consequent free radical formation. Another antioxidative mechanism was the donation of hydrogen to the radicals, which was crucial for protecting the lipids of cell membranes from autoxidation. Furthermore, a secondary antioxidative effect resulted from the binding of iron, copper, and other transition metals by ferulic acids, which prevented peroxidation of the cell membrane.[31] Recent studies have revealed the promising activity of umbelliprenin in the inhibition of inflammation, genotoxicity, carcinogenesis, acetylcholinesterase, and lipoxygenase, also demonstrating its cytotoxic effect.[32]

Antioxidant activity

Ferula assa-foetida resin extract exhibited strong DPPH radical scavenging activity from concentration as low as 15.625 µg/mL up to the highest concentration of 1000 µg/mL. At 31.25 µg/mL, the percentage radical scavenging activity exceeded 50%. At the concentration of 1000 µg/mL, the highest DPPH radical scavenging activity was recorded with a percentage radical scavenging of 96.5% against 98.3% for ascorbic acid at the same concentration (Figure 2). The IC_{50} for the radical scavenging activity of the extract was found to be 0.017 mg/mL. The total antioxidant capacity was calculated from the calibration curve of ascorbic acid ($y = 0.0022x - 0.0674$, $R^2 = 0.9949$). The total antioxidant capacity of the ethanol extract of *F. assa foetida* resin was found to be 3.31 mg AAE/g.

According to Bagheri *et al*. (2017),[33] the IC_{50} of antioxidant activity as measured by the DPPH radical scavenging assay of *F. assa foetida* oleo-gum resin was 109 mg/mL, while in another study by Bagheri *et al*. (2015), *F. assa foetida* oleo-gum resin exhibited an IC_{50} value for DPPH radical scavenging activity of 380 ± 12 mg/mL, whereas that of ascorbic acid, quercetin, and butylated hydroxyanisole (BHA) were 1.26 ± 0.11, 1.32 ± 0.07, and 13.49 ± 1.04 mg/mL, respectively.[34] The extract of the aerial parts of *F. assa foetida* have been shown to have good but different levels of antioxidant activity in different antioxidant models including ferrous ion (Fe^{++}) chelating, DPPH radical scavenging, and nitric oxide radical scavenging activities.[35] in addition *F. assa foetida* extract has shown a significant inhibition of lipid peroxidation as measured by thiobarbituric acid-reactive substances in the liver of rat.[36] The antioxidant potential of plant extracts is highly dependent on the presence of antioxidant molecules such as phenolic acids and flavonoids. Therefore, the variation in the antioxidant power of the extract obtained in the different studies could be explained by the differences in the content of polyphenols and particularly the nature of these compounds, which contributed significantly to their electron and hydrogen transfer capacity.[37]

Conclusion

The findings from the present study have shown that the ethanol extract of *F. assa foetida* resin possess varying degrees of antioxidant potential. The antioxidant activity of the extract could be attributed to the abundance of bioactive ingredients like phenols and flavonoids in the extract. Therefore oleo-gum resin has a great potential is natural source of antioxidant agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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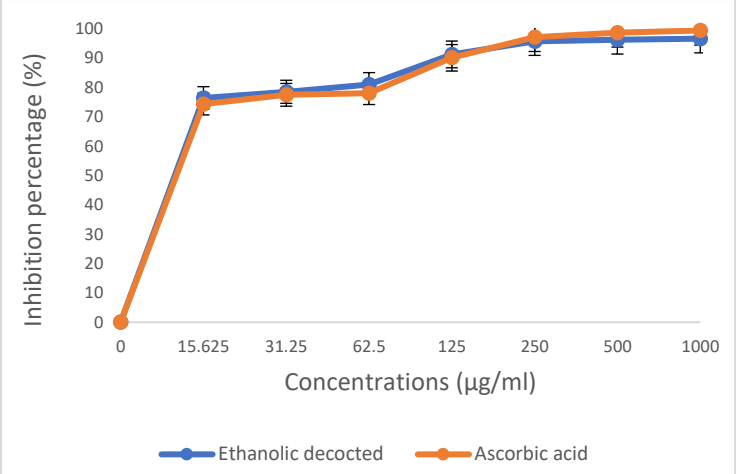

Figure 2: DPPH radical scavenging activity of *Ferula assa foetida* resin ethanol extract

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