Original paper

HPLC-DAD analysis and antioxidant potential of *Ferula assa foetida* resin ethanolic extract

Abstract:

Ferula assa foetida commonly consumed as a healthy beverage has been demonstrated to have various biological activities, including antioxidation. Our study aims to investigate the chemical composition and evaluation of the antioxidant effect of *F. assa foetida* resin ethanolic extract. First, the chemical composition of *F. assa foetida* resin ethanolic extract was determined using HPLC-DADESI-MS method. It was followed by phytochemical characterization of bioactive components. Then, the antioxidant potential was evaluated usinf the DPPH scavenging end total antioxidant methods. The decoction with ethanol has a yield of 33.22%. results of phytochemical screenig revealed the presence of various bioactive compounds with the remarkable presence of steroids, saponins, flavonoids, phenols, coumarins and cardiac glycosides. 11 compounds were identified in the ethanolic fraction: Vanilin acid, p-coumaric acid, Ferulic acid, 3,4-dihydroxybenzoic acid, Chlorogenic acid, Sinapic Acid, caffeic acid derivatived, Chrysin acid, Rosmarinic acid, Umbelliprenin and Galbanic acid. The DPPH free radical scavenging activity was recorded with a value of 96.5% against 98.3% for ascorbic acid. The ethanolic extract of *F. assa foetida* enregistred a value of 3,31 mg EAA/g. *Ferula assa foetida* resin manifested an interesant scavenging activity. Therefore, it can be considered as an alternative tresatment against oxidant stress and biomolecules damages.

Keywords: *F. assa foetida* resin, oxidant stress, polyphenols, extraction.

Introduction

Once regarded as worthless plant material, secondary metabolites are now considered as invaluable constituents having tremendous therapeutic potential (Delgoda and Murray, 2017). It is reckoned that naturally occurring secondary metabolites present in plants confer pharmacological properties that have been used by man since antiquity and is now the mainstream of drug development (Patridge et al. 2016). Several biochemical reactions in our

body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell et al. 1992). The harmful action of free radicals can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism **(**Zheng and Wang, 2001).

Researchers have aimed to find alternatives to replace synthetic antioxidants, with natural substances asserting their value as historically-accepted therapeutical agents (Wu et al. 2009). In fact, a major portion of the commonly used medical drugs is originally derived from natural substances (Shrivastava et al. 2012). Herbs have been identified as an important source of novel bioactive compounds for medicine development drugs (Balunas and Kinghorn, 2005). The efficacy of these herbal agents has been related with their antioxidant potential to reduce or inhibit free radical mediated damage to cellular macromolecules, such as DNA, lipids and proteins (Galati and O'Brien, 2004). *F. assa foetida* is used as an oleo gum resin (asafoetida) that obtained by incision of stem and root (Bagheri et al. 2010).

Recent pharmacological and biological studies have verified several pharmacological activities such as antioxidant, antileishmanial, anticonvulsant, anti-diabetic, antispasmodic, hypotensive and antinociceptive from this oleo gum resin (Bafghi et al. 2014; Bagheri et al. 2016). Therefore, the objective of the present study was to investigate the chemical composition and antioxidant properties of *F. assa foetida* resin ethanolic extract.

Materials and Methods

Plant material

The plant material was *Ferula assa foetida* resin imported from India and purchased from a grocer in the Wilaya of Mascara (Algeria). The resin has been ground into powder by a grinder, and the powder is stored in sealed glass bottles until used.

Preparation of ethanolic extract

3 g of resin of *Ferula assa foetida* was mixed with 60 ml of Ethanol and then heated until boiling. Then, the filtration was carried out on filter paper. The filtras obtained were evaporated using a rotary evaporator to recover the solvent then put them in an oven to remove the water. The extracts obtained were kept in the refrigerator after yield calculation until to their use.

Qualitative analysis

The phytochemical characterization of ethanolic extract of *F. assa foetida* was carried out by different classical methods based on coloration intensity and precipitation (Bagre et al. 2007; Mujeeb et al. 2014).

Quantitative analysis

Determination of total phenolic content

The dosage of total phenolic content was determined by spectrophotometry, according to the colorimetric method using Folin-Ciocalteu reagent (Wong et al. 2006). To 1.25 ml of Folin-Ciocalteu added 1ml of sodium carbonate NaCO3 % and 0.25 ml of extract (or standard substance of gallic acid and its dilutions). The mixture was incubated at room temperature for 90 min. The absorbance was measured at 765 nm. The results were expressed in mg gallic acid equivalent/g of dry plant matter (mg GAE/g dry) with reference to the gallic acid calibration curve.

Determination of flavonoid content

The flavonoids were measured using a method based on the formation of a very stable complex between aluminum chloride and oxygen atoms (Djeridane et al. 2006). 1ml of extract (or quercetin solution and its dilutions) was added to 0.3 ml of sodium nitrite (NaNO2) 5%. After 5 min, 0.3 ml of aluminum trichloride ($AlCl_3$) (10% in methanol) was added and after 6

min 2 ml of 4% sodium hydroxide (NaOH) was added and completed the volume to 10 ml. The absorbance was measured at 510 nm. The results were expressed in mg quercetin equivalent/g of dry plant matter by referring to the quercetin calibration curve.

Determination of tannins content

For the determination of tannins, the vanillin method with HCl was adopted. This method depended on the reaction of vanillin with the terminal flavonoid group of tannins and the formation of red complexes (Heimler et al. 2006). A volume of 0.5 ml of extract or standard (catechin) was added to the mixture of 3 ml of 4% (m/v) vanillin, 1.5 ml of hydrochloric acid then homogenized. The mixture was left to stand for 15 min at room temperature. The absorbance of each extract was measured at 500 nm using the UV-Visible spectrophotometer. The total content of condensed tannins was calculated as mg tannic acid equivalent (mg TAE/g) using the equation obtained from the calibration curve.

The identification of Polyphenols and Flavonoids by HPLC-DADESI-MS

Identification of *Ferula assa foetida* polyphenols and flavonoids was conducted on a Shimadzu-system (prominence I. LC-2030C 3D) equipped with a surveyor UVVIS diode array detection (DAD) and a LCQ advantage max ion trap mass spectrometer (all from Thermo fisher scientific, Waltham, MA, USA), coupled through an electrospray ionization (ESI) source. The separation was performed on ascentis express C18 column (15 cm x 4.6 mm) ID packed with 2.7 µm partially porous particles (Supelco, Bellefonte, PA, USA). The binary mobile phase consisted of water/acetic acid: 0.075% (solvent A) and methanol/acetic acid: 0.075% (solvent B). The gradient was 0-5 minutes: 2% of B, 5-80 minutes: 2%-100% of B, 80-85 minutes: 100%. The flow rate was 0.8 mL/min and the injection volume was 5 µl. PDA wavelength range was 190–400 nm and the chromatograms were extracted at 280 nm (time constant: 0.025 s; sample frequency: 40 Hz). MS acquisition was performed using an ESI interface, in the negative ion mode, under the following conditions: mass spectral range

100–800 m/z; interval: 0.5 s; scan speed: 1500 amu/s; nebulizing gas (N2) flow: 1.5 L/min; interface temperature: 350°C, heat block: 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. The results were obtained from the average of three determinations and are expressed as microgram per gram dried extract ± %RSD.

DPPH scavenging activity

The antiradical activity of the extract was determined according to the method of Sánchez-Moreno (2002). Practically, a quantity of 2 mg of DPPH was freshly prepared in 50 ml of methanol. A volume of 1.95 ml of DPPH was added to 200 µl of the solutions of the extracts and of the reference antioxidant (ascorbic acid) at different concentrations. The mixture was left in the dark for 30 min and the discoloration compared to the control containing only the DPPH solution is measured at 517 nm against a methanol blank. The negative control contained all the reagents except the test extract which was replaced by an equal volume of methanol. The reference antioxidant solution (ascorbic acid) represented the positive control. The percentage of antiradical activity was estimated according to the following equation:

$$\% PI = (Abs\ Control - Abs\ Extract)/Abs\ Control \times 100\%$$

%PI: Percentage of inhibition.

Abs Control: Absorbance of the negative control.

Abs Extract: Absorbance of the extract.

The study of the variation of the antiradical activity according to the concentration of the extracts makes it possible to determine the concentration which corresponds to 50% inhibition (IC50). A low IC50 value corresponding to a high efficiency of the extract.

Total antioxidant capacity (TAC)

The total antioxidant capacity is evaluated by the phosphomolybdenum method of Prieto et al. (1999). A volume of 0.3 ml of the extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were screwed on and incubated at 95° C. for 90 min. After cooling, the absorbance of the solutions was measured at 695 nm against the blank which contains 3 ml of the reagent solution and 0.3 ml of methanol and it was incubated under the same conditions as the sample. The total antioxidant capacity was expressed in milligram equivalents of ascorbic acid per gram of dry matter (mg EAA/g DM).

Results and discussion

Extraction yield

The extraction step from the resin of *Ferula assa foetida* generated an extract with a pasty appearance and brown or yellow in color. This is due to the presence of plant pigments or else to the nature of the polyphenols entrained by the solvent and to their solubility in this solvent. The decoction with ethanol has a yield of 33.22%. The extraction yield depends on the extraction method and also on the extraction solvent (Vazquez et al. 2008). Decoction remains effective for the extraction of phenolic compounds because it is an operation that should be reserved for the extraction of non-thermolabile active ingredients (Leybros and Frémeaux, 1990).

Qualitative analysis

The qualitative analysis of the extracts aims to highlight certain types of secondary metabolites in the resin. The detection of these chemical compounds is based on constituent solubility tests, precipitation reactions (intensity) and turbidity where the color is proportional to the quantity of the substance sought. The results of this screening carried out on the extract obtained are reported in the following table.

Table 1. Characterization of the chemical groups in the resin of *Ferula assa foetida.*

	Decocted Ethanolic
Steroïds	+++
Terpenoids	-
Tannins	+
Flavonoïds	+++
Alcaloïds	-
Saponins	+++
Phenols	+++
Anthraquinons	-
Glucoside cardiotonic	+++
Coumarins	+++

(-): Negative reaction.

(+): Weakly positive reaction.

(++): Moderately positive reaction.

(+++): Strongly positive reaction.

The preliminary phytochemical results carried out on the resin extract of *Ferula assa foetida* revealed the presence of various bioactive compounds with the remarkable presence of steroids, saponins, flavonoids, phenols, coumarins and cardiac glycosides. The tannins are weakly present. However, a total absence of terpenoids, anthraquinones and alkaloids is noted. In general, the extraction is influenced by the extraction methods chosen according to the phytochemicals to be studied. Other factors, such as pH, temperature and the ratio of the

quantity of matter to the volume of the solvent, also play an important role in the variation in the quantification of compounds. All of the chemical groups thus identified have remarkable pharmacological properties (Ouedraogo, 2001). The potential of a medicinal plant is attributed to the action of its phytochemical constituents which could justify its multiple therapeutic indications (Konkon et al. 2006).

Quantitative analysis

The concentrations of polyphenols and flavonoids of the Ferula-assa-foetida resin are determined from the calibration lines (y = 0.005x + 0.0137, R2 = 0.9982) and (y = 0.065x + 0.0854, R2 = 0.94) plotted using as standard gallic acid and quercetin respectively. The concentration is expressed in micrograms of gallic acid equivalent per milligram of extract (mg GAE/g of extract) for polyphenols and in micrograms of quercetin equivalent per milligram of extract (mg QE/g of extract) for flavonoids. Condensed tannin contents were expressed by reference to the catechin calibration curve (y= 0.997 x- 0.0003) with a coefficient of determination (R2 = 0.9473). The results are expressed in milligram (mg) equivalent of tannic acid per gram of dry plant matter (mg TAE/g DM). The quantification results revealed that the ethanolic extract of *Ferula assa foetida* presented interesting values in total polyphenols, flavonoids and tannins with values equal to125 mg GAE/g, 23,7 mg QE/g, 0,15 mg TAE/g respectively.

According to Yazdanipour et al. (2021), the total phenolic content value of the ethanolic extract 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) revealed that flavonoid content in leaf extract was 16.71 mg QE/g dried extract, while in gum extract it was only 0.11 mg QE/g dried extract. These results are in line with Ahmadvand et al. (2013). They reported that the flavonoid content in *Ferula assa foetida* leaf hydroalcoholic extract and Ferula asafoetida leaf essential oil were 12.53 mg/100 g and 0.015 mg/100 g, respectively.

Another study that measured the TPC of a species of Ferula, revealed that it contained 36.4 mg of phenolic components per gram of the extract (Ema et al. 1997). Phenolic and flavonoid compounds have considerable health-improving impacts and are essential elements in pharmaceutical and medicinal applications. This fact is ascribed to their anti-inflammatory, anti-cancer and anti-mutagenic aspects (Figg and Folkman, 2008).

The identification of Polyphenols and Flavonoids by HPLC-DADESI-MS

The chromatogram was presented in Fig. 1. A total of 11 compounds were identified in the ethanolic fraction: Vanilin acid, p-coumaric acid, Ferulic acid, 3,4-dihydroxybenzoic acid, Chlorogenic acid, Sinapic Acid, caffeic acid derivatived, Chrysin acid, Rosmarinic acid, Umbelliprenin and Galbanic acid (Table 2). In particular, the content of Ferulic acid appeared to be very higher (10,42%) compared to the other compounds followed by Rosmarinic acid (4,69%). These resultas were in agreement with those of Niazmand et al. (2021) who revealed that the phenolic profile of *F. assa foetida* leaf ethanolic extract identified 7 phenolic compounds including ferulic acid, vanilic acid, coumaric acid, umbelliprenin, galbanic acid, karatavicinol, and kamolonol. According to the results of Zengin et al. (2018), 42 compounds were tentatively identified from the alcoolic extract of *F. assa foetida.*

According to Yazdanipour et al. (2021), approximately 7 compounds having the highest relative abundance. Compared to the standards, the presence of active compounds of ferulic acid (21.55%), gummosin (3.39%), farnesiferol (6.57%), Galbanic acid (7.33%), samarcandin (11.35%), asafoetida (3.41%) and umbelliprenin (6.66%). Additionally, a comprehensive review published by Sgarbossa et al. (2015) illustrated the neuroprotective potential of ferulic acid as therapeutic agent in Alzheimer's disease. The review claimed the ability of ferulic acid to inhibit amyloid beta aggregations both in vitro and in vivo; modulate oxidative stress-induced apoptotic programmed cell death induced by oxidative stress. Membrane characteristics including charge, permeability, and physiochemical properties were

irreversibly modified by ferulic acid due to changes in hydrophobicity, generation of localized ruptures or pores, as well as a reduction in negative surface charge in the cellular membranes; essential intracellular contents consequently leaked out to the surrounding environment (Borges et al. 2013).

In terms of the underlying mechanism, ferulic acid's antioxidative activity is attributed to the reaction of the antioxidant molecule with a radical to form a stable phenoxyl radical, which impedes the initiation of complex reaction cascades and consequent free radical formation. Another possible antioxidative mechanism is the direct donation of hydrogen to the radicals, which is crucial for protecting the lipid acids of cell membranes from autoxidation. Furthermore, secondary antioxidative activity results from the binding of iron, copper and other transition metals by ferulic acids, which prevents peroxidation of the cell membrane due to hydroxyl radical formation (Zdun´ska et al. 2018). Studies conducted recently have revealed the promising activity of umbelliprenin in the inhibition of inflammation, carcinogenesis, genotoxicity, lipoxygenase, and acetylcholinesterase, also demonstrating its cytotoxic features (Shakeri et al. 2014).

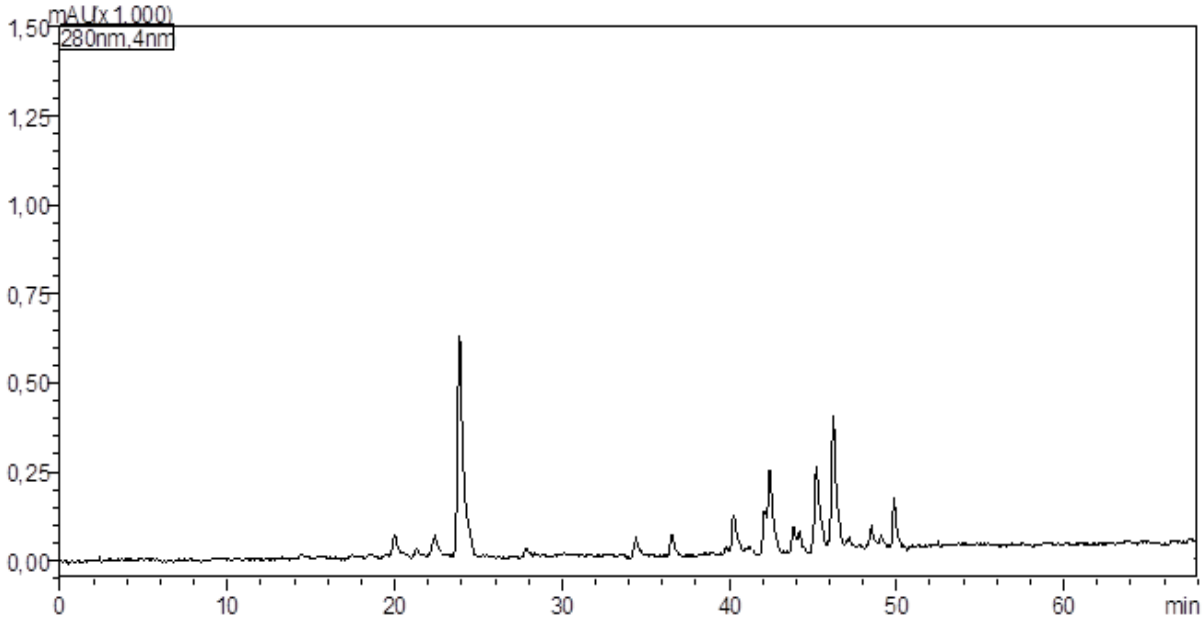

Fig. 1. Chromatogram of HPLC analysis

Table 2. Quantitative analysis of *F. assa foetida* resin ethanolic extract determined by HPLC.

Peak	RT	Compound	Area	% Area
1	20.006	Vanilin 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 derivatived	6432597	3.006
9	45.219	Chrysin acid	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

Antioxidant activities

The extract exhibited radical-scavenging activity from the concentration of 15.625 µg/ml. At 31.25 µg/ml, these percentages exceed 50%. At the concentration of 1000 µg/ml, the maximum DPPH free radical scavenging activity was recorded with a value of 96.5% against 98.3% for ascorbic acid at the same concentration (Fig. 2). The total antioxidant capacity of the different extracts is calculated from the calibration curve, established using ascorbic acid as reference antioxidant. This curve has the following formula as an equation: y= 0.291(x) – 0.520, with an R2=0.9875. The significant values of the absorbances obtained indicated the

strong antioxidant capacity of this standard molecule (ascorbic acid). The ethanolic extract of *F. assa foetida* enregistred a value of 3,31 mg EAA/g (Table 3).

According to Bagheri et al. (2017), results showed that the IC50 of antioxidant activity of *F. assa foetida* was 109 mg/ml. while the results of Dehpour et al. (2009) indicated that IC50 for DPPH radical-scavenging activity was 380 ± 12 mg/ml. The IC50 values for Ascorbic acid, quercetin and BHA were 1.26 ± 0.11, 1.32 ± 0.07 and 13.49 ± 1.04 mg/ml, respectively. The extract from aerial parts of *F. assa foetida* showed good but different levels of antioxidant activity in all the models studied. These extracts had good Fe++ chelation ability; DPPH and nitric oxide radicals scavenging activities. Further investigation of individual compound, determines various ways of antioxidant mechanisms involved (Upadhyay et al. 2017).

The importance of the antioxidant activity of plant extracts focused on their richness in molecules with high antioxidant potential such as phenolic acids and flavonoids. Indeed, according to the results obtained, the variation in the antioxidant power of the extract could be explained by their differential richness in polyphenols and more particularly the nature of these compounds which contributes to their electron transfer capacity / therefore d hydrogen (Benchaachoua, 2018). Asafoetida, administered orally to Sprague–Dawley rats at doses of 1.25% and 2.5% w/w, significantly restored the level of antioxidant system, depleted by N-methyl-N-nitrosourea treatment. There was a significant inhibition in lipid peroxidation as measured by thiobarbituric acid-reactive substances in the liver of rats (Mahendra and Bisht, 2012).

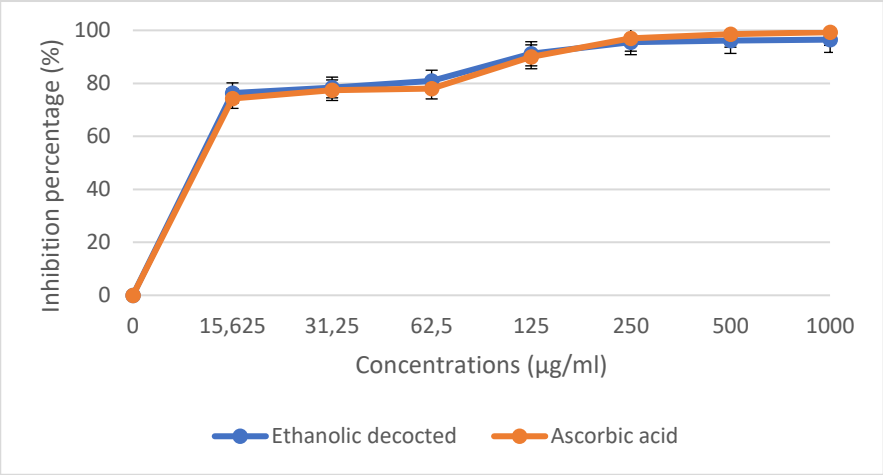

Fig. 2. Inhibition percentage (%) of the ethanolic extract of *F. assa foetida.*

Table 3. Antioxidant activities of ethanolic extract of *F. assa foetida.*

	IC50 (mg/ml)	TAC (mg EAA/g)
F. assa foetida.	0.017	3.31

Conclusions

The ethanolic extract of *F. assa foetida* resin exhibited different levels of antioxidant activity in all the both studied methods (DPPH scavenging and Total antioxidant capacity). It ca be presented as alternative drugs according to the richness of their extract with bioactive components.

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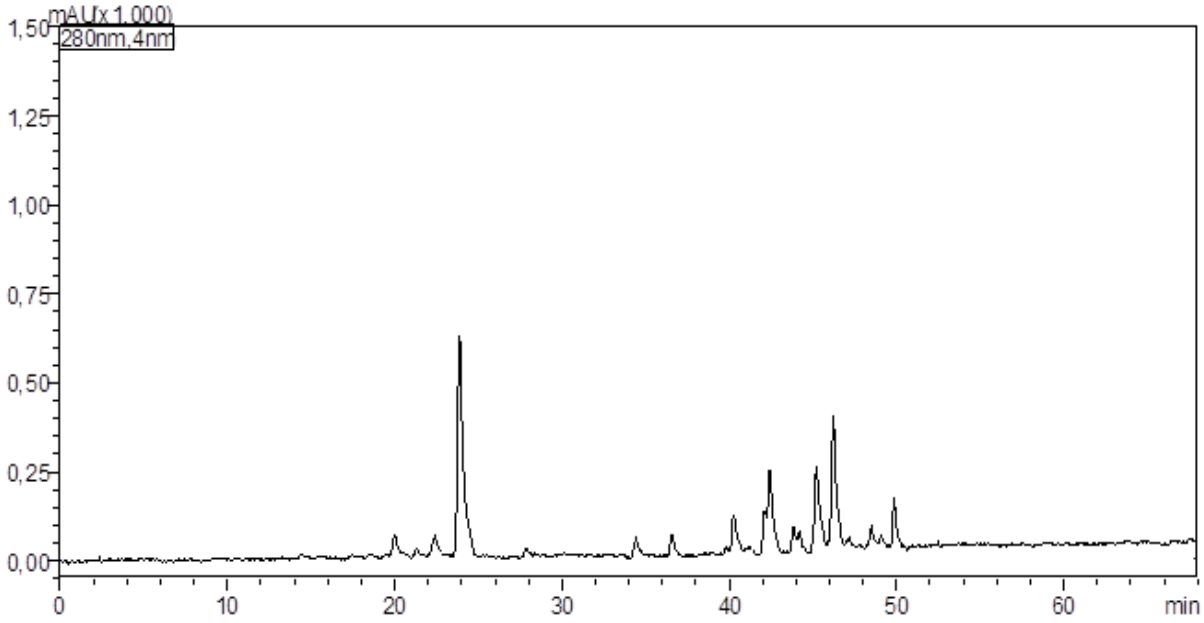

Fig. 1. Chromatogram of HPLC analysis

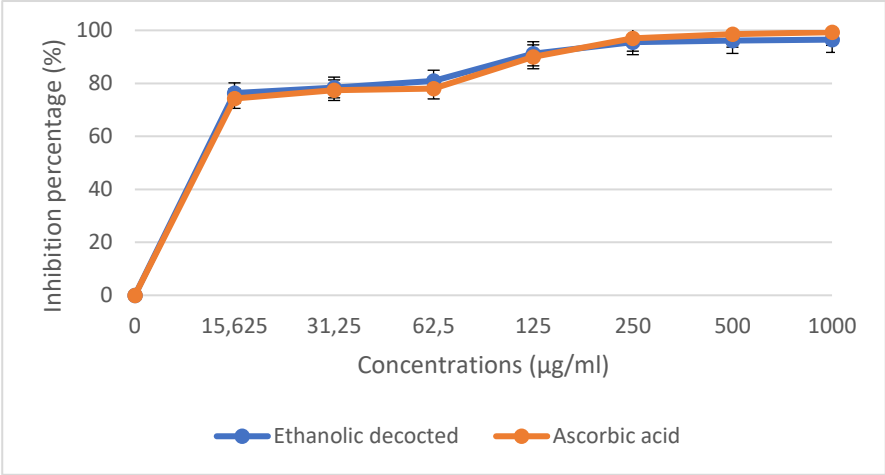

Fig. 2. Inhibition percentage (%) of the ethanolic extract of *F. assa foetida.*

Fig. 1. Chromatogram of HPLC analysis

Fig. 2. Inhibition percentage (%) of the ethanolic extract of *F. assa foetida.*