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Phytochemical Content, Antioxidant and Antifungal Activities of *Ephedra alata* Decne. Aerial Parts Grown Wild in South-west Algeria

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ABSTRACT

Background: The presence of antioxidants such as polyphenol, in plants may provide protection against a number of diseases. Moreover, pathogenic fungi can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock. The aim of present study was to determine the antioxidant and antifungal activities of aerial part extracts from *Ephedra alata* Decne.

Methods: Antifungal activities were tested against three pathogens fungi using mycelial growth, sporulation and germination spores.

Result: The determination of the polyphenol contents, total flavonoids and condensed tannins of the aerial part extracts gave respectively (TPC: 40.45±0.18 mg GAE/g, TFC: 133.25±0.11 mg QE/g, TCT: 20.76±0.19 mg CE/g). The flavonoids and methanolic extracts showed a good scavenging activity with an IC_{50}: 0.23±0.66; 0.54±0.35 mg/ml, respectively. For antifungal properties, flavonoids extract showed the inhibitoriest activity against fungi tested, followed by methanolic extract.

Key words: Antifungal activities, Antioxidant activity, *Ephedra alata* Decne., Polyphenol contents, Selective extracts.

INTRODUCTION

Fungal diseases cause considerable morbidity and mortality globally and elevate health care costs (Liu *et al*., 2019). *Aspergillus*, *Fusarium* and *Penicillium* and many other mycotoxin producers have become a major clinical problem and are the most important fungi affecting the yield of small grain cereal and causing spoilage of the derived foodstuffs (Salhi *et al*., 2017). The paucity of effective antifungal agents and emergency of drug resistance prompted researchers to identify novel antifungal agents.

Traditional medicinal plants have been an important source to discover new agents or lead compounds in the history of drug research and development (Liu *et al*., 2019). Increased consideration has been focused on the usage of natural antimicrobial agents from plant origins, due to their safety and efficacy as well as the fact that the majority of these plants are classified as generally recognized as safe (Suurbaar *et al*., 2017). Natural products provide the basis for the vast majority of anti-infective therapies currently in clinical use. For example, phenols (such as flavonoids, tannins), saponins and alkaloids are natural product derivatives and key classes of antifungal drugs and have antioxidant properties in the prevention and fight against oxidative stress (Da *et al*., 2019). For these reasons, global education has been conducted for the characterization, utilization and extraction of these biological and pharmacological active compounds from plant origins (Suurbaar *et al*., 2017).

Ephedra alata Decne. of the Ephedraceae family is a plant grows wildly on the gravely rocky, sandy and clay soil in arid environments often near shifting sand dunes, where Algeria is one from native land for this species. This plant is one of the most used medicinal plants in traditional medicine for the treatment of various diseases especially cancerous and respiratory diseases, also their stems are chewed for treatment of bacterial and fungal infections (Jaradat *et al*., 2021).

To date, the antimicrobial potential of some Ephedra species has been recognized, but there are few studies on its mode of antifungal action, especially against stage life of filamentous fungi: mycelial growth, sporulation and germination spores.

In this context, the main secondary metabolites classes, polyphenols content, antifungal properties of aerial part extracts from *E. alata* and its antioxidant activity were reported.

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MATERIALS AND METHODS

Experimental site

The study was carried at the Laboratory of Biology, University Tahri Mohammed, Bechar- Algeria in 2020.

Plant material

E. alata fresh aerial parts were collected in March 2020 from Taghit region, located 100 km from Bechar Department (South- West of Algeria). The plant was botanically identified and authenticated by the laboratory of botanics at Bechar University. The samples were washed, air-dried, grinded in a Wiley Mill to fine unifor mtexture and stored in glass jars until use.

Preparation of plant extracts

Approximate 20.0 g of dried plant powder was extracted at room temperature for 48 h with 100 mL of 75% methanol, then the extract was through filter paper. After filtration, the resultingsolution was evaporated under reduced pressure at 60°C. The residues were dried and weighed (6.5 g). The flavonoids were extracted using the method described by Lee *et al.,* (1995). The obtained residue was extracted with 100 mL of n-butanol, the n-butanolic phase was evaporated to dryness. The dry residue was extracted three times with 200 mL of the mixture (water/ethyl acetate) (v/v) for 1 hour. After separation and evaporation, the organic phase was dried, weighed (2.33 g) and stored at 4°C for further use. For tannins, the powder (100 g) were treated with 1 L of an aqueous solution of base ($NaHCO_3$ 0.5%, $NaHSO_3$ 2%). Samples were immersed in water under continuous magnetic stirring for 6 h at 100°C following the procedure developed by Dababi *et al.,* (2020). The raw tannin extracts were filtered using a fritted glass, dried in an oven at 50°C and weighed (0.33 g).

Phytochemical screening

The plant methanolic extract was screened for the presence of the phytochemical classes by using the standard methods explained by Khaldi *et al.,* (2019).

Total phenolic contents (TPC)

The extract (100 µL) and Folin-Ciocalteu reagent (100 µL) diluted (1/20) were added to 2 mL of 2% Na_2CO_3, mixed thoroughly and then incubated for 30 min in the dark at room temperature. The absorbance was then measured at 700 nm. Gallic acid was used as the standard and the TPC was expressed as mg of gallic acid equivalent (GAE)/g of dried extract (Chaouche *et al*., 2020).

Total flavonoid contents (TFC)

Extract (250 µL) is mixed with 75 µL of sodium nitrite (5 %) with as incubate for 6 min at room temperature, by adding 150 µL of aluminium chloride at 10%, after 5 min 1 M of sodium hydroxide solution (500 µL) was added to each extract and the final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm (Chaouche *et al.,* 2020).

Total condensed tannins (TCT)

500 µL aliquots of prepared extract were added to 3 mL of vanillin solution (4%) and 1.5 mL of concentrated sulfuric acid respectively, the mixture was allowed to stand for 15 min at room temperature (25°C) and the absorption was measured at 500 nm against solvent as a blank. The amount of TCT is expressed as mg (+)-catechin equivalents (CE)/g dry extract (Chaouche *et al*., 2020).

DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to Khaldi *et al.,* (2019). Ascorbic acid was used as common standard for antioxidant compound estimation. The result expressed in percentage of DPPH radical scavenging calculated according to the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where

A_0 = The absorbance of the control reaction.

A_1 = The absorbance in the presence of the sample extract.

The concentration of extract that caused 50% inhibition (IC_{50}) was calculated based on the graph of inhibition percentage plotted against extract concentration.

Antifungal activities

Microorganisms

The antifungal activities of different extracts were tested against three pathogens fungi, *Aspergillus flavus* MTTC 2799, *Penicillium expansum* MTTC 1344 and *Fusarium oxysporum f.sp. albedinis:* Foa, obtained from the laboratory of biology, Tahri Mohamed Bechar University. The strains were grown on potato dextrose agar acidified (PDAa) and incubated at 25°C for seven days in dark.

Mycelial growth assay

The minimum mycelial growth inhibitory concentration (MIC^m) of extracts was measured according to the contact direct method. Antifungal index of mycelial growth in percent (Im %) was performed according to Khaldi *et al.,* (2017), it was obtained by calculating the average of two perpendicular diameters (mycelial growth in control and mycelial growth of the test).

Sporulation assay

The minimum sporulation inhibitory concentration (MIC^s) of extracts was performed as described by Khaldi *et al*., (2017). The percentage of inhibition was calculated using the following formula:

$$\text{(Is \%)} = \frac{(N_0 - Nc)}{N_0} \times 100$$

Where

N_0 = The number of the spores estimated in control.

Nc = The number of the spores estimated in the presence of extract.

Spore germination assay

The minimum germination inhibitory concentration (MIC^g) of extracts was evaluated as determined by (Khaldi *et al*., 2017)

The percentage inhibition of germination (Ig %) was determined by the following formula:

$$(Ig\ \%) = \frac{(N_0 - Nc)}{N_0} \times 100$$

Where

N_0 = The number of germinated spores in control.

Nc = The number of germinated spores in the presence of extract.

Statistical analysis

All the measurements were replicated thrice for each treatment and the data reported as mean±standard deviations. Signifficant differences between the mean values were determined by Duncan's test ($P<0.05$ and $P<0.01$). The Pearson rank correlation test was used for comparisons between the broth dilution and methods used for antifungal activities.

RESULTS AND DISCUSSION

Alkaloids, reducing compounds, steroid, terpenoids, phenols, flavonoids and tannins were revealed to be present in methanolic extract of *E. alata*. However, saponosids, anthraquinones, glucosides and coumarins were not detected. Our results are consistent with those of Jaradat *et al.,* (2021) except for steroid and terpenoids and in harmony with those reported by Benarba *et al.,* (2021) except for alkaloids, saponosids and coumarins. The metabolites identified in this plant were some of the most important phytochemical classes.

The TPC, TFC and TCT of methanolic, flavonoid and tannin extracts is calculated from the calibration curve ($R^2=1$), were 40.45±0.18 mg GAE/g, 133.25±0.11 mg QE/g and 20.76±0.19 mg CE/g, respectively. The analysis indicated *E. alata* collected from the Algerian Sahara (Bechar) had medium TPC, higher TFC and lower TCT. The aerial parts from this plant is used for the treatment of various diseases, so it is necessary to estimate the content of phenolic compounds in the this part extracts and these compounds are considered responsible of different biological activities. The results from TPC of our extracts are higher to those reported by some recent studies of Jaradat *et al.,* (2021) and Benarba *et al.,* (2021), but from TFC and TCT are higher to those reported by first authors and lower to those indicated by second authors. In comparison of Tunisian *E. alata*, our species had lower TPC and higher TFC (Ibragic and Sofić, 2015). This difference may be due to various factors such as the geographical location and date, physiological stage of plant, heterogeneity of genetic resources, environmental adaptation, soil properties, rainfall, plant storage and extraction methods (Benarba *et al*., 2021).

The scavenging capacity of 1 mg doses of methanolic, flavonoids and tannins extracts found to be 0.54±0.35, 0.23±0.66 and 2.02±0.77 mg/ml, respectively (Table 1). The IC_{50} of the ascorbic acid used as a standard was found to be (0.12±0.25 mg/ml). The *E. alata* flavonoids extract had higher antioxidant potentials compared to the methanolic and tannin extracts which could be explained by its higher TFC. Our results are consistent with those reported by Benarba *et al.,* (2021) and Jaradat *et al.,* (2021) revealed a better antioxidant activity with lower IC_{50}, which can be attributed to their phenolic and flavonoid contents, however, its opposite with those reported by Jaradat *et al.,* (2015) attested that of Palestinian *E. alata,* can be attributed to their alkaloids content. In general, the phenolic compounds of all extracts and especially flavonoids extract was considerably in significant quantity, which could be a major contributor to the strong antioxidant effect of *E. alata* aerial parts. It is well known that phenolic compounds play a crucial role on the free radical scavenging and reduction of oxygen concentration, or protection and regeneration of other antioxidant molecules (Aici et Benmehdi, 2020).

Antifungal activities of the three fungi species are shown in Table 2. The results indicated that mycelia growth was considerably reduced with increasing concentration of *E. alata*. From this table, flavonoids and methanolic extracts have significant antifungal activity against two pathogens fungi. Flavonoids extract had good inhibitory activity against *P. expansum* and *A. flavus* at $MICs^m$ as weak as 1500 and 3000 μg/ml respectively, followed by methanolic extract inhibited the *P. expansum* at MIC^m as from 3500 μg/ml, while tannins extract had no good inhibitory activity. *Foa* proved to be the most resistant for all extracts, this is quite clear by comparing the inhibitory percentages.

On spore sporulation, all strains were not inhibited completely, the strongest inhibitory activities were observed against *P. expansum* and *A. flavus* with flavonoids extract at high dose (90.13±0.45% and 80.52±0.31%) respectively. On the other side, the both extracts showed, *in vitro*, important antifungal activity against germination, flavonoids extract exhibited the highest effect and inhibited the *P. expansum* and *A. flavus* at $MICs^g$ as weak as 900 and 1500 μg/ml respectively, whilst for methanolic extract, *P. expansum* and *A. flavus* were inhibited at $MICs^g$ as from 1800 and 3000 μg/ml,

Table 1: Total phenolic, tannins, flavonoid contents and antioxidant activities of plant extracts.

Extract	TPC[a] mg of GAE/g extract	TCT[a] mg of CE/g extract	TFC[a] mg of QE/g extract	$IC_{50}^{a,b}$ (mg/mL)
Methanolic	40.45±0.18	-	-	0.54±0.35
Tannins	-	20.76±0.19	-	2.02±0.77
Flavonoids	-	-	133.25±0.11	0.23±0.66
Ascorbic acid	-	-	-	0.12±0.25

[a] Means with different letters were significantly different at the level of $p<0.05$.

[b] Lower IC_{50} values indicated the highest radical scavenging activity. '-' not determined.

Table 2: Antifungal activities of plant extracts expressed in (%).

C	Extract	*A. flavus*			*P. expansum*			*Foa*		
		Im	Ig	Is	Im	Ig	Is	Im	Ig	Is
Control	M	0	0	0	0	0	0	0	0	0
	T	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0
250	M	1.08±45	23.17±50	0	12.45±0.15	22.53±0.51	3.80±0.72	0	0	0
	T	0	0	0	0	5.14±0.35	0	0	0	0
	F	10.02±0.12	19.40±0.87	3.72±0.83	20.05±0.15	45.24±0.73	8.25±0.29	0	4.75±0.33	0
500	M	8.10±0.35	44.86±0.63	2.33±0.84	28.25±0.70	58.76±0.25	14.33±0.50	0	1.25±0.25	0
	T	0	5.23±0.01	0	2.06±0.28	11.14±0.35	0	0	0	0
	F	25.15±0.25	42.39±0.13	16.26±0.61	40.38±0.25	71.72±0.32	22.50±0.62	2.65±0.55	9.90±0.67	0
1000	M	20.13±0.46	65.44±0.12	11.66±0.92	53.15±0.23	81.45±0.35*	40.25±0.60	1.26±0.36	8.84±0.43	0
	T	6.12±0.35	11.19±0.41	0	10.65±0.58	20.60±93	5.05±0.15	0	1.27±0.53	0
	F	50.19±0.85	76.15±0.08	38.13±0.95	75.95±0.70	100**	55.75±0.12	10.12±0.28	15.52±0.35	3.35±0.50
2000	M	41.15±0.34	88.05±0.37*	30.58±0.73	82.01±0.45*	100**	60.85±0.35	10.24±0.57	15.30±0.65	2.85±0.63
	T	15.30±0.25	21.22±0.15	4.75±0.55	20.25±0.40	36.46±0.51	14.73±0.35	5.45±0.31	9.39±0.12	0
	F	80.70±0.62*	100**	60.77±0.03	100**	100**	72.24±0.08	20.11±0.47	26.34±0.41	15.29±0.41
4000	M	70.75±0.51	100**	60.65±0.32	100**	100**	80.05±0.22*	20.19±0.48	30.23±0.42	10.40±0.75
	T	25.17±0.47	35.43±0.31	15.26±0.54	38.16±0.80	50.26±0.75	25.29±0.74	10.15±0.28	19.26±0.88	1.45±0.36
	F	100**	100**	80.52±0.31*	100**	100**	90.13±0.45*	40.07±0.38	45.19±0.73	30.25±0.57
MIC	M	nd	3000	nd	3500	1800	nd	nd	nd	nd
	T	nd	nd	nd	nd	nd	nd	nd	nd	nd
	F	3000	1500	nd	1500	900	nd	nd	nd	nd

* Significant at $p<0.05$; ** Significant at $p<0.01$ according to controls; C: Concentration (µg/ml); M: Methanolic; T: Tannins; F: Flavonoids; Im: Antifungal index of mycelial growth; Ig: Percentage inhibition of germination; Is: Percentage inhibition of sporulation; MIC: Minimal inhibition concentrations; nd: Concentration not detected. Each value in the table was obtained by calculating the average of three experiments±standard deviation.

respectively. By contrast, the most interesting inhibition of tannins extract was observed against *P. expansum* at high concentration (50.26±0.75%).

Inhibition in mycelial growth is generally associated with germination and sporulation inhibition. However, our results revealed that flavonoids and methanolic extracts reduce or even inhibit the mycelial growth and germination according to extract concentration without showing the same total inhibition when associated with sporulation.

The antifungal activity of our extracts can be explained on the basis of their polyphenolic contents and the fungus metabolism. Polyphenols have previously been reported to have a wide spectrum of biological activity, including antimicrobial activity (Zhou *et al.,* 2015). Redondo-blanco *et al*., (2020) reported that a phenolic acid involved in plant growth development, has shown antifungal properties against postharvest pathogens, including *P. expansum*, even at low concentrations. Other studies have been also proved that phenolic compounds effectively inhibit fungal proliferation of *A. flavus* (Zhou *et al.,* 2015). In contrast, some authors have reported that *F. oxysporum* was affected by the polyphenol extract, but is not really the case in the present study (Redondo-Blanco *et al.,* 2020).

In the same context, the high antifungal activity in the flavonoids extract may be due to the presence of high amount TFC. Previous studies indicate that flavonoids of several plant extracts showed antifungal activities, it play important roles in the development of plants and in the defence against aggressive fungus (Abdelkebir *et al*., 2018). Flavonoids inhibit fungal spore germination and have been proposed to control fungal pathogens (Redondo-Blanco *et al*., 2020).

The tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and they may assume pharmacological importance (Salhi *et al*., 2017). In several cases that may well be true, but that is not the case in this study and this can be explained by lower TCT. It also stated that, some authors hypothesized that they act by mitigating oxidative stress on the fungus (Molyneux *et al*., 2007).

CONCLUSION

This study confirms that the aerial parts of *E. alata* from South-West Algeria contain important phytochemical classes, specifically phenolic compounds. It is rich in flavonoids. Due to the presence of those compounds, their extracts have important antioxidant and strong antifungal activities mainly against mycelia growth and germination. As a traditional Algerian medicine, *E. alata* represents a promising herbal medicine for the discovery of novel antifungal compounds and can be developed as radical scavenging agents.

Conflict of interest: None.

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