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Phenolic and flavonoid contents and antioxidant activity of *Tourneuxia variifolia* Coss. *extracts* (Asteraceae)

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Abstract

In this study, the ethyl acetate and n-butanol extracts of the north africa endemic species Tourneuxia variifolia Coss. Were used to evaluate the antioxidant activity using DPPH radical scavenging activity, ABTS˙ decolorization, Cupric reducing power and reducing power in vitro essays and their phenolic content were also measured. The amounts of total phenolic and flavonoid contents were higher in ethyl acetate extract (979.5±3,53 mgGAE and 182±2.32 mgQE, respectively). However; EtOAc extract possessed a strong antioxidant activity in DPPH, ABTS and reducing power tests (IC_{50} =13.8±0.33; 5.18± 0.37; 23.85 ±2.88) whereas CUPRAC test (IC_{50} =204± 2.47µg/ml).

Keywords: Tourneuxia variifolia; antioxidant activity; phenolic content; flavonoid content.

1. Introduction

Reactive oxygen species may cause serious damage in the body as they are known to be highly responsible for the oxidation of biological molecules; such as lipids, DNA, and proteins (1). Oxidative stress is closely related to many degenerative diseases, such as diabetes, cancer, cardiovascular and neurodegenerative diseases (2). In such cases, antioxidant defense of the body needs to be supported by dietary antioxidants. Besides antioxidant vitamins, fruit and vegetable consumption (3).

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potential. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins (4).

The Asteraceae family is one of the largest flowering plant families, with over 1600 genera and 25000 species worldwide. The Asteraceae family members show a wide range of anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective activities (5). Many species of Asteraceae demonstrate various pharmacological activities, which have been attributed to their phytochemical components, including essential oils, lignans, saponins, and polyphenolic compounds (6).

There are some less well-known species, as is the case of T*ourneuxia variifolia,* the new endemic species from South Africa (7). A recent study showed that EtOAc and n-BuOH extracts have potent anticancer properties. These properties were associated with the presence of powerful flavonoids and phenolic acids detected and identified by the LC/MS-MS method, such as quercetin, isoquercitrin, astragalin, rutin, and protocatechuic acid (8), which were characterized by their biological properties, particularly as potent antioxidants. The current work aimed to investigate for the first time the phenolic contents and antioxidant activity of ethyl acetate and *n*-butanol extracts from *T. variifolia* Coss. using *in-vitro* tests.

2. Material and methods

2.1 Plant material

Tourneuxia variifolia was collected during the flowering phase in March 2015, in the southeast of Algeria, and was identified by Mr. Benadelhakem (Director of the protection of nature agency, Bechar, Algeria). An authenticated voucher specimen (ATV 03/15) was deposited at the Herbarium of the VARENBIOMOL unit research frères Mentouri university of Constantine, Algeria.

2.2 Extraction

The aerial parts (1200g) were macerated three times for 72 hours in a hydroalcoholic mixture (MeOH/H_2O 80:20). After filtration, the crude extract was concentrated at room temperature and diluted with distilled water. The remaining aqueous solution was extracted with EtOAc and *n-BuOH,* respectively. Both extracts were filtered, dried, and then concentrated to give 6g of EtOAc and 36g of *n*-BuOH extracts.Total bioactive compounds.

2.3 Antioxidant activity

2.3.1 Total phenolic and flavonoid contents

The total phenolic content (TPC) was determined using Folin–Ciocalteu reagents using gallic acid as standard (Singleton et Rossi., 1965)[9]. 100µL Folin–Ciocalteu phenol reagents was added to 20µL of extract or standard solution. After 5 minutes, 20% sodium carbonate (75 µL) was added to the mixture. After being kept in total darkness for 2h, the absorbance was measured at 765nm. The results were expressed as gallic acid equivalents (GAE)/g of dry plant matter.

The total flavonoids (TFC) were determined according to the Folin-Ciocalteau colorimetric method with slight modifications (Topçu et al., 2007) [10]. 50 µL of extract (1mg/ml), 130 µL of MeOH, 10 µL of CH3COOk and 10 µl of Al(NO3)2, 9H2O) was added and mixed all together. After standing for 40 min, the absorbance of the solution was measured at 430 nm with a spectrophotometer. The results were expressed in mg quercetin/g dry weight by comparison with the quercetin standard curve.

2.3.2 Determination of 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

The antioxidant activity of the DPPH assay was established by Blois in 1958 [11]. In the radical form, DPPH had a deep violet color in the absence of phenolic compounds, and it became pale yellow in the presence of antioxidant products, followed by a decrease in its absorbance. In a 96-well plate, 160µl of DPPH solution at 0.1 mM was mixed with 40µl of ethyl acetate and *n*-buthanol extracts solutions at various concentrations. The plate was kept in the dark at room temperature and the absorbance was measured at 517 nm after 30 min of incubation. BHA, BHT, and ascorbic acid were used as standards. The scavenging activity of the extract was calculated using the following equation:

$$\text{DPPH effect (\%)} = \left(\frac{Acontrol - Asample}{Acontrol}\right)*100$$

Where $A_{control}$ and A_{sample} are the absorbances of the reference and sample obtained from the UV-visible spectrophotometer respectively.

2.3.3 Determination of 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid scavenging activity

The $ABTS^{\bullet+}$ scavenging activity was evaluated according to Re et al., 1999 [12]. 160µL of potassium persulfate (2.45mM) and diluted $ABTS^{\bullet+}$ solution (7 mM ABTS in water) were mixed and diluted in water to obtain an absorbance of 0.708±0.025 at 734 nm. 160 µL of the mixture was added to 40µL of extracts dissolved in methanol at different concentrations. After 10 min of incubation, the absorbance was measured at 734nm. Each assay for all samples was carried out in triplicate. BHA, BHT, and ascorbic acid were used as standards. The percentage inhibition of all samples was calculated using the above equation.

2.3.4 Cupric reducing antioxidant capacity

The CUPRAC was determined according to Apak et al.,2004 [13]. 50µl of $CuCl_2$ solution (10mM) followed by 50µl of neocuproine solution (7.5 mM), 60µl of NH_4Ac buffer solution (1M, pH7.0) and 40µl of the Ethyl acetate or *n*-BuOH solution at different concentrations; those volumes were put into a 96 well round-bottomed plate, the absorbance was read at 450nm, after 60 min incubation. Results were given as $A_{0.5}$, corresponding to the concentration indicated as 50 % absorbance intensity compared with the absorbance of BHA, BHT, and ascorbic acid, which were used as standards.

2.3.5 Reducing power

the reducing power of the previously mentioned extracts was determined according to Oyaizu (1986) [14]. 40 µL of 0.2 M phosphate buffer (pH 6.6), 50µL of potassium ferricyanide (1%) as well as 10µL of sample at various concentrations, were mixed and incubated at 50°C for 20 min. After cooling, 50µL of trichloroacetic acid (TCA) (10%) was added, and the mixture was centrifuged for 10min. Then; 40µL of the later solution was mixed with 40µL of distilled water plus 10µL of ferric chloride (0.1%). The absorbance was measured at 700 nm.

Statistical analyses

All the experimental results are mentioned as a mean ± standard deviation of three trials.

3. Results and discussion

3.1 Total phenolic-flavonoid content

The total phenolic and flavonoid contents of ethyl acetat and n-butanol extracts of T. variifolia species were investigated using the Folin-Ciocalteu and Aluminium chloride methods respectively.

Table 1 shows that, the contents of total phenolics and flavonoids considerably varied between both extracts and ranged from 979,5±3,53 to 895.66±2.22 µgGAE/mg extract for total phenolic, and from 182.66±2.32 to 151.44±1.66 µgQE/mg extract for total flavonoid. The highest amounts of both total phenolic and flavonoids were found in EtOAc extract, followed by *n*-BuOH extract.

Table1: phenolic-flavonoid contents of *T.variifolia* extracts

Extracts	TPC (µg GAE/mg extract)	TFC (µg QE/mg extract)
AcOEt	979.5±3,53	182.66±2.32
n-BuOH	895.66±2.22	151.44±1.66

The amounts of total phenolics and flavonoids varied considerably between the two extracts. The highest amounts of both total phenolics and flavonoids were found in EtOAc extract (979.5±3,53µgGAE/mg extract; 182.66±2.32 µgQE/mg extract), followed by the *n*-BuOH extract (895.66±2.22 µgQE/mg extract; 151.44±1.66 µgQE/mg extract), the phenolic components were found to be higher than the flavonoid components.

3.2 Antioxydant activity

In this study, the antioxidant activity of both EtOAc and *n*-BuOH extracts of *T. variifolia* was compared to BHT, BHA, and ascorbic acid. These comparisons were performed by various methods such as DPPH free radical scavenging, cupric reducing antioxidant capacity, ABTS cation radical decolorization assays, and reducing power (table 2).

As indicated in table 2, the EtOAc and *n*-BuOH extracts exhibited strong antioxidant activity in all tested methods. Although both extracts showed promising activity (IC_{50}= 13.8±0.37 and 19.75±3.95 µg/ml) in the DPPH free radical scavenging assay, the ethyl acetate extract exhibited a comparable activity with the ascorbic acid (IC_{50}=13.94±2.81 µg/ml). As seen in table 2, in the ABTS cation radical scavenging assay, both extracts showed very strong activities (IC_{50}= 5.18±0.37 and 15.95±5.44 µg /ml). In addition; both EtOAc and *n*-BuOH extracts showed moderated activities ($A_{0.5}$=23.85±2.88; 39.6±0.57 µg/ml) and ($A_{0.5}$=204±2.47; 262±7.72 µg/ml) in CUPRAC and reducing power methods, respectively.

This high antioxidant activity of *T. variifolia* extracts might be due to the high total phenolic and flavonoids contents as well as the presence of quercetin, astragalin, *p*-coumaric acid, and protocatechuic acid with high amounts in the EtOAc extract detected and identified by the LC/MS-MS method as indicated previously. This may have contributed to their high antioxidant activity, which reflects their potent antioxidant agents [15-20].

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4. Conclusion

The current paper deals for the first time with the antioxidant activity of the aerial parts of *Tourneuxia variifolia* extracts and their phenolic contents. This study shows that EtOAc and *n*-BuOH extracts have potent antioxidant properties. This may be due to the presence of phenolic compounds in the extracts. The evaluation of the antioxidant effects of *T. variifolia* extracts leads to the conclusion that *T. variifolia* could be identified as a potential plant exhibiting antioxidant properties.

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Table2: Antioxidant activity of *T. variifolia* extracts

	DPPH	ABTS	Reducing power	CUPRAC
	IC_{50} (µg/ml)		$A_{0.5}$ (µg/ml)	
AcOEt	13.8±0.33	5.18±0.37	23.85±2.88	204±2.47
n-BuOH	19.75±3.95	15.95±5.44	39.6±0.57	262±7.72
BHA	6.14±0.41	1.81±0.1	7.99±1,87	5.35±0.71
BHT	12.99±0.41	1.29±0.3	-	8.97±3.94
Ascorbic acid	13.94±2.81	1.74±0.1	6.77±1.15	12.43±0.09

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