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Decoction, Infusion and Ethanolic Extract of *Juncus acutus* Rhizome: Phytochemical Content and Antioxidant Properties

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ABSTRACT

In order to find out new sources of safe and inexpensive antioxidants, infusion, decoction and ethanolic extract of Juncus acutus rhizome were analysed for their antioxidant activities (AA) using ABTS, DPPH radicals cation, and ferric reducing /antioxidant power (FRAP) methods. The total phenolic (TPC), total flavonoid (TFC) and condensed tannins (CTC) contents were measured. The results indicated that the total phenolic contents ranged from 52.12 ± 0.90 to 133.72 ± 0.09 mg gallic acid equivalent/g of extract. Results showed that the ethanol extract has the highest TPC, TFC and CTC compared to other extracts. The most active extract towards the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the highest radical scavenging activity and reducing power was obtained in the ethanol extract. This study suggests that Juncus acutus may serve as a good source of natural antioxidants.

Keywords: *Juncus acutus*, Total phenolic, Total flavonoid, Condensed tannins, Antioxidant activities.

INTRODUCTION

There is ample evidence that reactive oxygen/nitrogen species generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer [1,2]. However, there is wide spread agreement that some synthetic antioxidants such as butylhyroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants due to their potential health risks and toxicity [3,4]. Therefore, it is very important to find out new sources of safes and in expensives antioxidants of natural origin. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [5].

Juncus Acutus (J. acutus) (*Juncaceae*) commonly known as" Smar", could be one of those matrices, providing a wide range of biomolecules, such as phenolic compounds. *Juncus acutus* is a perennial plant that belongs to the genus *Juncus*, with about 315 species, is one of the largest genera in the Monocotyledons. The *genus* is almost cosmopolitan in distribution, but uncommon in the tropics and absent from Antarctica. The major centers of diversity of the genus are western north America, the mediterranean region of Europe, the Sino-Himalayan region, the Far East, South Africa and South-Eastern (SE) Australia/New Zealand [6]. The nutritive value of *J. acutus* has been studied in terms of chemical composition, organic matter digestibility (OMD) and metabolizable energy (ME) [7] using the *in vitro* gas production method [8-10]. Consequently, it has been proposed as an alternative roughage source for ruminants [7]. The species *J. acutus* is traditionally used for the treatment of infection and inflammation [11] and is endowed with antioxidant compounds, such as phenolics (e.g., 8, 8′-bidehydrojuncusol) and flavones (e.g., luteolin), which were isolated from methanol extracts of the rhizomes [12]. But as far as we know no reports are available in decoctions, infusion and ethanolic extract of *J. acutus* rhizomes. In the present work, we collected rhizomes of *J. acutus*, prepared its water and ethanolic extracts, and analyzed its antioxidant activities and polyphenols, flavonoids and tannins contents.

MATERIALS AND METHODS

Sample preparation

The rhizome of *J. acutus* was collected from Gabès (south of Tunisia) on July 2014. The plant sample was air-dried in shade, reduced to a fine powder, packed in tightly closed containers, and stored for phytochemical and biological studies.

Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH),6-hydroxy-2,5,7,8-tetramethylchromancarboxylic acid (Trolox), 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), gallic acid and Folin–Ciocalteu's phenolic reagent were obtained from Sigma–Aldrich Co. (St. Louis, France).

Preparation of the alcoholic extract, infusion and decoction

Alcoholic extraction was performed by stirring the plant material (10 g) with 180 mL of ethanol at 25°C for 7 days and filtered through Whatman No. 4 paper. The extract was evaporated at 35°C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and then conserved until use.

Infusion were prepared by adding 200 mL of boiling distilled water to the sample (1 g) and were left to stand at room temperature for 5 min, and then filtered under reduced pressure. Decoction were performed by adding to 200 mL of distilled water to the sample (1 g), heated (heating plate, VELP scientific) and boiled for 5 min. The mixtures were left to stand for 5 min and then filtered under reduced pressure.

Total phenolic content (TPC) determination

TPC were estimated according to the method of [13] Briefly Folin Ciocalteu reagent (5 mL, diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL) were added to the extract solutions (1 mL), vortexed for 15 s and allowed to stand for 30 min at 40°C. The absorbance was measured at 765 nm and the results were expressed as mg of gallic acid equivalent per g of extract.

Total flavonoid content (TFC) determination

TFC were determined according to the method [14]. An aliquot (250 µL) of the extract or standard solution was mixed with 1250 µL of dd H_2O and 75 µL of 5% $NaNO_2$ solution. After 6 min, 150 µL of 10% $AlCl_3$. H_2O solution were added. After 6 min, 0.5 mL of 1 M NaOH solution were added and then the total volume was made up to 2.5 mL with distilled H_2O. After mixing of the solution, the absorbance against blank was determined at 510 nm. The results were expressed as mg catechine equivalents (CE)/ g of extract.

Condensed tannins content (CTC) determination

CTC were determined according to the method of Julkunen et al., [15]. An aliquot (50 µL) of the extract was mixed with 1.5 mL of 4% vanillin (prepared with methanol) and then 750 µL of concentrated HCl were added. The well mixed solution was incubated at ambient temperature in the dark for 20 min. The absorbance against blank was read at 500 nm. The results were expressed as mg of catechin equivalent (CE) per milligram of extract.

Antioxidant activity

Scavenging capacity of ABTS radical cation

Antioxidant activity (AA) was measured using an improved ABTS method as described by Re et al., [16]. The ABTS radical cation ($ABTS^{\cdot+}$) solution was prepared through the reaction of 39.2 mg ABTS and 6.7 mg potassium persulphate, and incubated at 23°C in the dark for 24 h. The $ABTS^{\bullet+}$ solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.002 at 734 nm. 3.9 mL of $ABTS^{\bullet+}$ solution was added to 0.1 mL of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23°C for 6 min and the absorbance at 734 nm was immediately recorded.

All determinations were carried out in triplicate. The percent absorbance reduction was determined as follows:

$$\% \text{ ABTS inhibition} = [Abs_b - Abs_f / Abs_b] \times 100$$

where Abs_b is the absorption of blank sample (t = 0 min) and Abs_f is the absorption of tested extract solution (t = 6 min). IC_{50} values are referred to the extract concentration at the 50% of the antioxidant activity.

Scavenging capacity of DPPH radical

DPPH Radical scavenging activity of extracts was measured following the method of Brand-Williams et al., [17] with slight modifications. The solution of DPPH· in methanol (60 µM) was prepared daily, before UV measurements. 90 µl s of the extract were mixed with 810 µl of 0.3 mmol L^{-1} solution of DPPH in methanol, incubated in the dark for 30 min and the absorbance of the mixture was monitored at 517 nm. The decreasing of the DPPH· solution absorbance indicated an increase of the DPPH radical-scavenging activity. The experiment was carried out in triplicate. Radical scavenging activity (%) was calculated by the following formula:

$$[(A_{dpph} - A_s)/ A_{DPPH}] \times 100$$

Where A_S is the absorbance of the solution containing the sample at 517 nm, and A_{dpph} is the absorbance of the DPPH solution. IC_{50} values are referred to the extract concentration at the 50% of the antioxidant activity.

Determination of total antioxidant activity by Ferric Reducing/Antioxidant Power (FRAP) assay

The ferric reducing power of plant extracts was determined using a modified version of the FRAP assay [18]. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% ***w/v***, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% ***w/v***, 0.5 mL) was added. Then 0.8 mL of deionized water, 0.16 mL of ferric chloride (0.1%) was added to 0.8 ml of the mixture and the absorbance was measured at 690 nm. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as a standard.

Statistical analysis

All parameters were determined in triplicate for each sample. Statistical analysis was performed with SPSS (11.0). Analysis of variance (ANOVA), multivariate and Tukey tests were applied. Duncan's test ($\boldsymbol{p} < 0.05$) was used to determine significant differences between means.

RESULTS AND DISCUSSION

Total polyphenol, flavonoid and tannin contents of infusion, decoction and ethanolic extract of J. acutus rhizome

Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological activities such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity [19]. Total polyphenol, flavonoid, and tannin contents of the water and ethanol extracts of *J. acutus* rhizome are shown in Table 1. TPC content is expressed as mg of galiq acid equivalent per milligram of extract. The extract with the highest TPC was the ethanol extract (133, 72 ± 0.09 mg eq GA/g E). By comparison between the total phenols values given in different reports, our results are higher than those reported for the arial part extracts of *Juncus gerardii* (3.39 ± 0.27 mg eq GA/g) [20].

For the water extracts, TPC content of the *J. acutus* rhizome ranged from 52,12 ± 0,90 mg to 58,50 ± 0,73 mg eq GA/g E for decoction and infusion respectively. It appears that the vast majority of polyphenols are not water soluble. Koffi et al. [21] reported that water extraction of plant organs leaves a large amount of residual polyphenols that only an appropriate combination of solvents would extract. Therefore to be assured of obtaining fractions rich in polyphenols manufacturers would have to use

extraction solvents with a mixture of suitable solvents [22]. Total flavonoid content was expressed as mg of catechin equivalent (CE) per milligram of extract. The ethanol extract attained the highest significant value of flavonoid (142.21 ± 3.47 mg eq CE/g E). However, for the water extract, infusion of *J. acutus* rhizome exhibited the highest value of flavonoids (53.88 ± 1.42 mg eq CE/g E). Ragarding the results reported by Erdem et al., [23], *J. acutus* rhizome showed 15-fold and 8-fold higher amount of flavonoid than the stems and seeds ether extract. The flavonoid-phenolic ratio in Table 1 is mentioned to show the importance of flavonoids in total phenolic content and its antioxidant activity. The range of this ratio is between 0.92 for infusion and 1, 06 in ethanol extract. The CTC content was expressed as mg of rutin equivalent per milligram of extract. As shown in Table 1, ethanol extract of Juncus rhizome had the highest CTC (91, 20 ± 5.98 mg eq CE/g E) followed by the infusion (26,91 ± 2,9 mg eq CE/g E) and then the decoction (8,22 ± 1,24 mg eq CE/g E).

Table 1: Extraction yield (%), Total phenol, flavonoid and condensed tannin contents of infusion, decoction and ethanolic extract of *J. acutus* rhizome.

	Infusion	**Decoction**	**Ethanolic Extract**
Extraction yields	10.69 ± 0.09	17.46 ± 0.05	19.22 ± 1.51
TPC (mg eq GA/g E)	58.50 ± 0,73b	52,12 ± 0,90a	133,72 ± 0.09c
TFC (mg eq CAT/g E)	53.88 ± 1,42b	48,66 ± 0,72a	142,21 ± 3.47c
CTC (mg eq CAT/g E)	26.91 ± 2,99b	8,22 ± 1,24a	91,20 ± 5.98c
Total flavonoid/Total phenolic	0.92	0,93	1,06
Note: The total phenolic (TPC), total flavonoid (TFC) and condensed tannins (CTC) contents, In each row different letters mean significant differences ($p < 0.05$).			

Antioxidant activity

The antioxidant capacities of the plant extracts largely depend on the composition of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action [24].

Scavenging capacity of DPPH radical

Previous studies have indicated a key role for free radicals as major contributors to aging and to degenerative diseases of aging, such as immune system decline, cancer, brain dysfunction, and cardiovascular disease [25], so free radical scavenging activity is of great importance. DPPH can make a stable free radical in aqueous or ethanol solution. Upon receiving proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and became yellow. Table 2 shows the antioxidant capacities of *J. acutus* rhizome infusion, decoction and ethanolic extract using the DPPH assays. The extracts, in general, showed high antioxidant capacities. The most active extract towards the DPPH radical was the ethanol extract with an IC_{50} value of 0.04 mg/ml. Our results are better than those reported by Maria et al., [26] for the methanol extract of *J. acutus* (IC_{50} = 0.4 mg/mL), and the diethyl ether extracts of *J. acutus* (IC_{50} = 0.2 mg/mL) arial parts. As for infusion and decoction of *J. acutus* rhizome, those extracts are the most active extracts towards the 1,1-diphenyl-2-picrylhydrazyl (DPPH) by comparison with the aqueous extract of the arial part of five halophytes from southern Portugal (*Arthrocnemum macrostachyum, Mesembryanthemum edule, Juncus acutus, Plantago coronopus and Halimione portulacoides*) as previously reported by Maria et al., [26].

Scavenging activity on ABTS radical

ABTS radical cation decolorisation assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain breaking antioxidants [27]. This radical has a relatively stable blue-green color, which is measured at 734 nm. Antioxidants in the extracts reduce intensity of this color to a degree that is proportion to their antioxidant concentration or activity. As shown in Table 2, the lowest IC50 values were obtained in the ethanol extract (IC_{50}= 0.24 mg/ml) followed by infusion and decoction respectively. The extract with the highest total phenolic content (TPC) was the ethanol extract 133.72 mg gallic acid equivalents (GAE)/g DW), which was correlated with a high radical scavenging activities (RSA). This is in agreement with several reports of positive correlations between the content of phenolics of halophyte extracts and its capacity to scavenge free radicals [28-30]. However, in other extracts and species no correlation between these parameters was observed, similar to the findings of Conforti et al., [31] in hydroalcoholic extracts of mediterranean dietary plants. This suggests that the RSA of these samples might be due to combined action of phenolic compounds with other components such as peptides and organic acids [32].

Antioxidant activity by the Ferric reducing antioxidant power: (FRAP) method

Antioxidants can be explained as reductants, and the inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in their action medium as reducing ability [33]. FRAP assay of the examined extracts was shown in Table 2. There was significant difference between different extracts in FRAP activity. In agreement with findings from the ABTS and DPPH assays, the IC_{50} value measured by FRAP assay was significantly lower in ethanolic extract by comparison with decoction and infusion. Regarding previous studies, *J. acutus* rhizome extracts had the highest value of reducing capacity compared with the methanol (IC_{50} = 0.26 ± 0.05) and water extract (IC_{50}= 0.56 ± 0.01) of *J. effuses* [34].

Table 2: Antioxidant activity (IC_{50} values, mg/ml) of infusion, decoction and ethanolic extract of *J. acutus* rhizome (mean ± SD)

Extract	**Infusion**	**Decoction**	**Ethanolic**
DPPH	0.364 ± 0.03b	0.419 ± 0.03c	0.04 ± 0,00a
ABTS	0.522 ± 0.02b	0.633 ± 0.04c	0,24 ± 0,002a
FRAP	0.397 ± 0.007b	0.453 ± 0.01c	0,11 ± 0,003a
Note: DPPH:2,2-difenyl-1-picryl hydrazyl; ABTS:2,2 –Azinobis (3-ethylbenzothiazo-line-6-sulphonic acid) diammonium salt; FRAP: ferric reducing/antioxidant power; IC50 values correspond to the sample concentration providing 50% of antioxidant activity. In each row different letters mean significant differences ($p < 0.05$).			

CONCLUSION

In conclusion, the results from these *in vitro* experiments, including ABTS radical monocation scavenging , DPPH radical scavenging , reducing power method, total polyphenol content, total flavonoid content and total condensed tannin , demonstrated that *J. acutus* rhizome infusion , decoction and ethanolic estracts had a high phenolic content and a potent antioxidant activity. Further study is needed to isolate and identify the antioxidative components of those extracts.

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