Effect of ionizing radiation on antioxidants and antibacterial activities of *Inula Viscasa*

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تأثير الإشعاع المؤين على المواد المضادة للاكسدة والأنشطة المضادة للبكتيريا للأخذرية

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١. وحدة البحوث وتطبيق التقنيات النووية في مجالات الصحة والزراعة، والبيئة، المركز الوطني للعلوم والتكنولوجيا النووية سيدي ثابت التكنولوجي و ٢٠٢٠ أريانة، تونس
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الملخص:

تم في هذا البحث التطرق الي مدي تاثير الاشعة المؤينة بجرعة قيمتها ٥ كيلو جراي علي النبتة الأخذرية . و قد تم استعمال تقنيات ABTS DPPH وFRAP لقياس الجذور الحرة. كما تم التأكد من مدي تاثير الاشعة المؤينة الا نشطة المضادة للبكتيريا لمكونات هذه النبتة. ويبين هذا البحث أنه على الرغم من انخفاض النشاط المضاد للأكسدة اثر التشعيع فان مكونات هذه النبتة قد حافظت على خصائصها المضادة للبكتيريا، يشير هذا بحث إلى إمكانية استخدام أشعة غاما كتقنية آمنة للحفاظ على النباتات الطبية مع أنشطة فعالة مضادة للبكتيريا و المضادة للأكسدة

Abstract:

In the present study, the irradiation processing of Tunisian *Inula Viscosa* samples was carried out at dose of 5 kGy. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity, the azinobis ethylbenzothialzoline 6-sulphonic acid (ABTS) Ferric reducing antioxidant power (FRAP) assay and the antibacterial activities of both control and irradiated samples extracted in methanol and ethanol were evaluated. The results showed that the irrTunisian *Inula Viscosa* extracts had strong antioxidant ability. The scavenger DPPH, ABTS and FRAP values of all extracts decreased significantly after irradiation. In addition, all extracts were effective against all the gram positive and gram negative pathogens. Gamma irradiation preserved the antibacterial activities of extracts and enhanced significantly ($p<0.05$) the activity of extracts against E.coli. These data indicated the potential use of gamma-irradiation as a safe technique for preservation of *Inula Viscosa* as a medicinal plant with effective antioxidant and antibacterial activities.

1. Introduction

Inula Viscosa is an herbaceous plant, belongs to family of Asteraceae. This weed is widely distributed over the Mediterranean region [1], where it flowers in late summer and beginning of autumn. It grows on hillslopes, damp habitats, and roadsides [2]. In Tunisian flok medicine it is used for treatment for many diseases such as digestive disorders, dietary supplements, and diabetes.

I. viscose is used as an herbal source for fungicidal preparations against foliar diseases belonging to the families Oomycetes, Ascomycetes, and Basidiomycetes [2].

Over the last decades studies have demonstrated that the crude extracts of *Inula Viscosia* have a strong anti-inflammatory effect, Antioxidant, antiseptic, antiphlogistic and anti-insecticidal activities [3, 4, 5].

Recents researchs were intensively investigated in order to purify and identify the bioactive compounds of *Inula Viscosa* such as sesquiterpene acids, sesquiterpene lactones, flavonoids and polyphenols [6, 7].

Antioxidants scavenge free radicals are one of the most important ways to prevent intracellular oxidative damage, such as cancer, atherosclerosis, aging, and other degenerative diseases [8, 9]

Many studies have revealed that the majority of the antioxidant activity may be from biochemicals such as flavonoids, anthocyanins, catechins and other phenolics.

In the flora of Tunisia, numerous plants belonging to the family *Asteraceae* have been reported to characterize their antioxidant and radical scavenging activities. However, no scientific reports on the antioxidant components or in vitro properties of *Inula Viscosa* from Tunisia have ever been published. The aim of this work is to study the effect of ionizing radiation on antioxidants properties of *Inula Viscosa* extract from Tunisia by DPPH, ABTS and FRAP assays. Additionally, antibacterial extracts have been determined.

2. Materials and methods

2.1.Chemical and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, FeCl3, 2,4,6-tripyridyl-S-triazine (TPTZ), potassium erricyanide [K3Fe(CN)6], BHT and trolox were purchased from SigmaAldrich Inc. (Steinheim, Germany). Solvents of analytical and HPLC grade were purchased from Carlo Erba Reactif-CDS (Val de Reuil, France).

2.2. Plant material

Leaves and flowers of *Inula viscosa* were collected from were randomly collected in November 2014 from wild population, in sidi thabet Ariana Tunisia. The plant material was botanically characterized by Prof. Nadia Ben Brahim (Laboratoire de botanique et des plantes d'ornement, Institut National de Recherche Agronomique de Tunis),

2.3. Preparation of extracts

Powder of flowers and leaves of *Inula viscosa* were defatted with hexane then extracted with methanol and ethanol by maceration in a flask and stirring in a water bath shaking incubator for 24 hours. The extraction solution was filtered, concentrated under reduced pressure in a Heidolph rotary evaporator (Schwabach, Germany) and dried. Then the extract were obtained and stored at 5 C for the following experiment.

2.4. Gamma -Irradiation

The Tunisian gamma irradiation facility (at Sidi Thabet) is designed for the preservation of foodstuff and sterilisation of medical devices. The source consists of eight encapsulated 60Co pencils with a diameter of 9.7 mm and an overall length of 452 mm. The starting activity of the source was 99.162 kC. These sources are stored in dry conditions in a cylindrical shield container in which they were transported. *Inula viscosa* leaves and flowers samples were exposed to gamma radiation at 5 kGy at a dose rate of 22.21 Gy/min and at room temperature (27 ± 2 C).

2.5. Antioxidant activity of extracts

2.5.1. DPPH radical scavenging assay

The DPPH radical scavenging activity of *Inula viscosa* extract was determined according to the method described by Scherer and Godoy [10] with a slight modification. Briefly, 1ml of methanolic solution containing different amounts of 0.01–1 mg/ml were mixed with 2ml of methanolic DPPH solution of DPPH (0.1 mM).

The mixture was vortexed and incubated in the dark at ambient temperature for1hour.

The absorbance was then measured at 517nm.

Radical scavenging activity was calculated as followed:
I(%) = $[(A_0 - A_1)/A_0]$ *100 where A_0 was the absorbance of control and A_1 was the absorbance of the sample. The result was expressed in the effective concentration of extract needed to get to I 50% (EC50).

2.5.2. ABTS radical scavenging activity

The ABTS radical scavenging activity method is based on the ability of molecules to scavenge the ABTS radical cation. ABTS activity was determined according to the method of Re [11]with slight modification. Briefly, ABTS (7 mM) solution was prepared according to the method previously reported by Pan [12]. The ABTS radical cation was diluted with methanol for an initial absorbance of about 0.70±0.02 at 745 nm. Extract solution (0.15ml) at different concentration were mixed with ABTS solution (2.85 ml), then absorbance was read at ambient temperature after 15 min.

The mixture was left to stand at room temperature in the dark for 15 min, and then the absorbance was measured at 734 nm. Trolox was used as positive control. The antioxidant capacity of test samples was expressed as EC50.

I(%) = $[(A_0 - A_1)/A_0]$ *100 Where all symbols have the same meaning as in the DPPH assay

2.5.3. Ferric reducing antioxidant power (FRAP)

The FRAP assay measures the ability of the antioxidants in the sample extracts to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the blue colored ferrous form (Fe^{2+}) can be monitored at 593 nm. The ferric-reducing antioxidant power was determined according to Benzie & Strainn [13]. The working FRAP reagents included 300 mM acetate buffer pH 3.6, 40 mM hydrochloric acid, 10 mM TPTZ solution and 20 mM ferric chloride solution. The FRAP solution was incubated at 37 °C for 30 min.

A sample (150 µL) was mixed with 2850 µL of FRAP solution and kept for 30 min in the dark. For quantification, a calibration curve of Trolox was prepared with dilutions from 0.1 mM to 1mM. . The antioxidant activities are expresses as mmol Trolox equivalent per gram dry weight amaranth sample (mmol AAE/g DW).

2.6. Antimicrobial activity

The antimicrobial activity of the extracts was determinate by the paper-disk diffusion method (NCCLS, 1997). Tested organisms consisted on two Gram(−) bacteria: E. coli(ATCC8739) and S.typhymurium (ATCC 14028) obtained from the Institut Pasteur, Paris, France, two Gram (+) bacteria E feacium, S. agalactiae isolated in the Institut National des Sciences Appliquées de Tunis, Tunisia.

Pure colonies were transferred to a saline solution the bacterial suspension was compared to a standard 0.5 Mc Farland which must be stirred before use (its absorbance should be between 0.08 to 0.1 at 625 nm, standard 0.5 corresponds approximately to 10^8 CFU / ml). The tested suspension microorganism was spread on the solid Mueller–Hinton Filter Filter paper disks (6 mm in diameter) were drenched with 15µl of alcoholic extracts and placed on the inoculated plates. After being kept at 4 C for 2 h, they were incubated at 37 C for 24 h. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

2.7. Statistical analysis

All measurements were carried out in triplicate and the results were presented as mean values ± SD. Statistical analyses were performed using a one-way analysis of variance ANOVA test and the significance of the difference between means was determined by Duncan's multiple range test. Differences at $P< 0.05$ were considered statistically significant.

3. Results and discussion

3.1. DPPH scavenging activity

DPPH free radical losses absorption after accepting an electron from samples which have hydrogen-donating abilities.

As shown in Table 1, all extracts exhibited excellent DPPH scavenging activity. The values were affected extraction solvent. It was higher for methanol than for ethanol. An increase in DPPH scavenging ability was observed with increase in concentration of extracts as shown in figure (1).

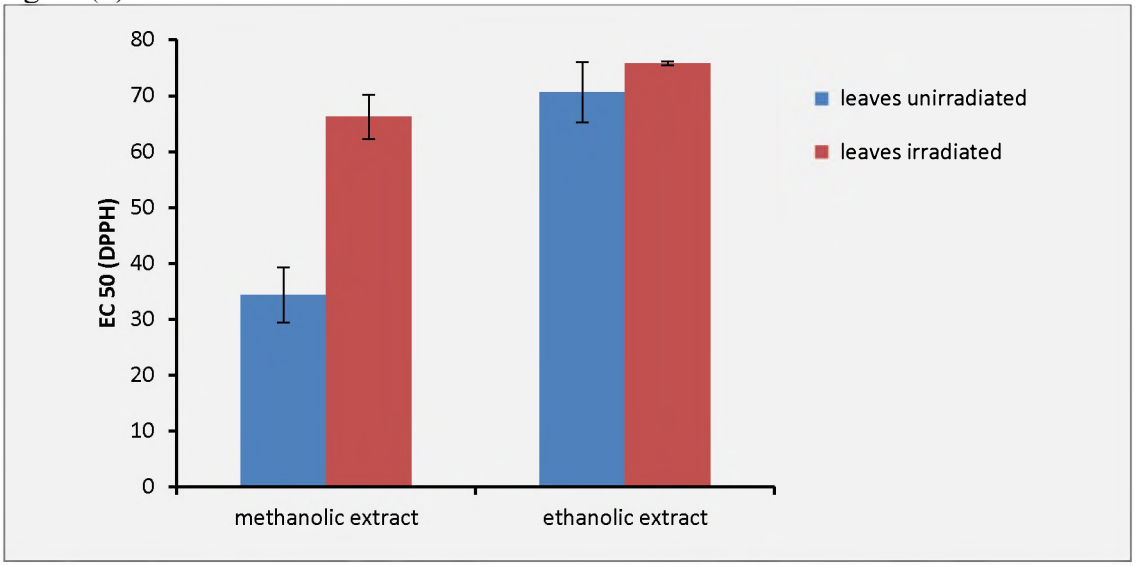

Fig.1. EC 50 of DPPH for the different concentrations of leaves methanolic extract control and irradiated compared with Trolox.

Table1: Effect of γ-irradiation on the DPPH radical scavenging activities of different *Inula Viscosa* extracts

	DPPH Radical scavenging activities EC50 (µg/mL)			
Irradiation dose (kGy)	methanolic leaves	ethanolic leaves	methanolic flowers	ethanolic flowers
0	34,39±4,939	70,586±5,394	44,321±4,827	54,1± 2,383
5	66,228±3,964	75,775±0,367	70,198±2,134	99,578±2,749

The EC50 parameter was used to compare the power of antioxidant activity .A low EC50 value states a strong antioxidant activity. It was found that the methanolic leaves extract proved much stronger DPPH radical scavenging activities with EC50 values of 34,39±4,939 µg/mL, followed by methanolic flowers (EC50= 70,198±2,134 µg/mL) , ethanolic flowers extract (EC50= 54,1± 2,383 µg/mL)and ethanolic leaves (EC50=70,586±5,394 µg/mL) respectively. These results are comparable with those of Trimech [14] who reported that the flowers (EC50=0.21±0.05 µg/mL) of *Inula viscosa* were the most powerful antioxidants in the DPPH test than leaves (EC50=0.26 ±0.05 µg/mL). Many researches demonstrated a positive correlation between antioxidative properties and phenolic content. They reported that the total polyphenols had major contributors to the antioxidant activities of plant materials [15].

After 5kGy irradiation, the extract remains active against the DPPH free radical. However the power of scavenging revealed a decrease significantly ($p > 0.05$) after irradiation extracts. This result is also supported by previous studies, Koseki [16] demonstrated alterations in the active principles in dehydrated herbs following increasing doses between 10 and 30 kGy. On the other hand, Byun [17] showed that the antioxidant electron donating ability of Korean medicinal herbs treated by gamma irradiation at 10 kGy did not differ from that of the nonirradiated control. Then Pérez [18] applied 30 kGy dose to dry sage and oregano for sanitization which did not significantly affect the capacity to inhibit the DPPH radical or the reducing power of the methanolic and aqueous extract. However, Pérez [19] indicated that

the irradiation treatment at 30 kGy increased 22% of EC50 values in the antioxidant activity of ethanol and water extracts from rosemary but the treatment had no significant effect on the methanolic extract.

3.2. ABTS scavenging activity

The ABTS assay is based on the generation of a blue/green $ABTS^{+}$ that can be reduced by antioxidants. It is an excellent index reflecting the antioxidant activity of the sample [20]. Table.2 and Figure (2) shows appreciable levels of antioxidant activity in the ABTS test.

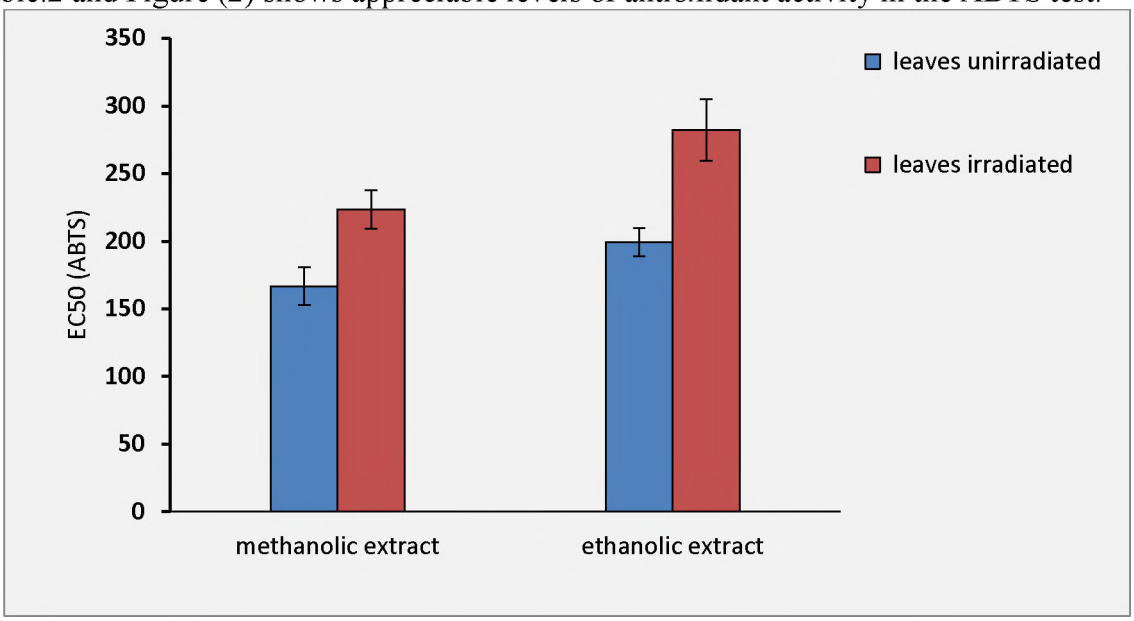

Fig.2. EC50 of ABTS for different concentrations of leaves methanolic extract control and irradiated compared with Trolox.

Table2: Effect of γ-irradiation on the ABTS radical scavenging activities of different *Inula Viscosa* extracts

	ABTS radical scavenging activities EC50 (µg/mL)			
Irradiation dose (kGy)	methanolic leaves	ethanolic leaves	methanolic flowers	ethanolic flowers
0	66,691±13,95	199,322±10,359	220,1976±20,046	289,978±12,621
5	223,391±14,101	282,351±22,866	234,191±0,758	273,829±9,328

The scavenging activity of samples from methanolic extracts were significantly ($p < 0.05$) higher (EC50 =66,691±13,95µg/mL for leaves and EC50 =234,191±0,758/mL for flowers) than ethanolic extracts (EC50 =282,351±22,8665µg /mL for leaves and EC50 =273,829±9,3285µg /mL for flowers). ABTS test confirms the strong reducing power ability of extracts from Tunisian *Inula viscosa* .

After irradiation at 5kGy, all extracts remained active against the radical however a statistically significant decrease ($p<0.05$) of the reducing power of extracts (fig.2). It should be noted that the gamma irradiation caused chemical modifications on many components such as flavonoid components and phenolic acid [21]. This fact may provide an explanation for the decrease in the antioxidant activity of extracts because of the measured positive relationships between phenolics content and antioxidant activity [22].

Ahn [23] also reported that the scavenging ability of Chinese cabbage was reduced after irradiation at 2 kGy.

3.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} at low pH by electron-donating antioxidants, resulting in an absorbance increase at 593 nm [24]. This trend was almost the same as that of the DPPH radical assay. A significant differences were noticed

between both extracts depends on the solvent ($p < 0.05$). Leaves possessed the highest reducing power leaves methanolic extracts followed by flowers methanolic extracts ($p < 0.05$) is shown in Table. 3.

Table 3: Effect of γ-irradiation on the ferric reducing activities of different *Inula Viscosa* extracts

	The ferric reducing ability Eq trolox(µm/ml)			
Irradiation dose (kGy)	methanolic leaves	ethanolic leaves	methanolic flowers	ethanolic flowers
0	0,931±0,019	0,722±0,030	0,778±0,017	0,690±0,0469
5	0,793±0,019	0,566±0,035	0,700±0,022	0,601±0,021

After gamma irradiation at 5kGy, all extracts kept their power reduction of Fe^{3+} but a statistically significant decrease ($p<0.05$) of the reducing power was observed. This result could be explained by the fact that irradiation induced variation of the components. According to Breitfellner [21], the irradiation treatment noticeably diminished some flavonoids, catechin and kaempferol components, in strawberries materials.

For *Inula Viscosa*, there is no study about the influence of the gamma irradiation over the antioxidant activity, but the effect of gamma irradiation on caffeic acid, the major compound of polyphenol *Inula viscosa,* was investigated. Krimmel [25] proved that the irradiation initiated the release of caffeic acid from chlorogenic acid as well as from rosmarinic acid. Another explanation was cited by Alothman [26] that the antioxidant concentration in a plant produce could be related to the duration of storage.

Aouidi [27] found that the gamma irradiation treatments have been shown to either increase or decrease the total phenolics content of plants materials, which is dependent on the dose delivered and the raw material used.

3.4. Antimicrobial activity

The anti-bacterial activity of leaves and flowers of *Inula Viscosa* against four bacterial species is summarized in Table (4.5.6.7).

Table.4. Diameter of microbial inhibition zone (mm) of leaves methanolic extract determined by Disk diffusion method

Dose (kGy)	Escherichia coli ATCC 8739	Salmonella typhimurium ATCC 14028	Enterococcus feacium ATCC 19434	Streptococcus agalactiae
0	9±0.1	10±0.2	15±0.1	22±0.3
5	11.5±0.2	7.8±0.3	16±0.1	10±0.1

Table.5. Diameter of microbial inhibition zone (mm) of leaves ethanolic extract determined by Disk diffusion method

Dose (kGy)	Escherichia coli ATCC 8739	Salmonella typhimurium ATCC 14028	Enterococcus feacium ATCC 19434	Streptococcus agalactiae
0	9±0.2	12±0.3	17±0.8	20±0.2
5	11±0.1	11.5±0.5	21±1	12±1

Table.6.Diameter of microbial inhibition zone (mm) of flowers methanolic extract by Disk diffusion method.

Dose (kGy)	Escherichia coli ATCC 8739	Salmonella typhimurium ATCC 14028	Enterococcus feacium ATCC 19434	Streptococcus agalactiae
0	11±0.2	11±0.3	20±0.5	24±1
5	11±0.5	8±0.1	15±1	15±0.2

Table.7.Diameter of microbial inhibition zone (mm) of flowers ethanolic extract determined by Disk diffusion method

Dose (kGy)	Escherichia coli ATCC 8739	Salmonella typhimurium ATCC 14028	Enterococcus feacium ATCC 19434	Streptococcus agalactiae
0	8±0.1	10±0.1	12±0.3	15±0.2
5	13±0.3	10.5±0.6	20±0.5	19±0.3

The results in terms of zone of inhibition (mm) showed that all extracts were effective against Gram positive (E feacium, S. agalactiae) and Gram-negative bacteria (E. coli and S. typhimurium). The extracts of Tunisian *Inula viscosa* residues were found to be more effective against two Gram-positive bacteria then Gram negative. The resistance of Gram-positive bacteria towards plants extracts has been previously attributed to the presence of membrane consisting of lipoproteins and lipopolysaccharides, which is selectively permeable and thus regulates access to the underlying structures [28]. The antibacterial potency of the extracts would be related to the main components such as carvacrol and thymol which are phenolic monoterpenes [29].

The antibacterial properties of *Inula Viscosa* were not affected by irradiation at 5 kGy. Gamma irradiation has different effects on the activity of extracts depending on the solvent and bacteria. However irradiation significantly ($p<0.05$) increases the activity of extracts against E.coli.

Conclusion

The present study was carried out to explore antioxidant and antimicrobial potential of methanol and ethanol extracts of *Inula Viscosa.* Ionizing radiation is known to stimulate the generation of oxygen radicals which destabilize organic molecules resulting in a decrease of the system's antioxidant potential. This research showed that gamma irradiation till 5 kGy affected negatively the antioxidant activity however the extracts still showed an excellent antioxidant activity. The irradiated and unirradiated methanol extracts exhibited a higher level of scavenging activity and a higher phenolic content, whether they were irradiated or no irradiated.

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