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Chemical investigation of different extracts and essential oil from the tubers of (Tunisian) *Cyperus rotundus*. Correlation with their antiradical and antimutagenic properties

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Abstract - The mutagenic potential of aqueous, Total Oligomers Flavonoids (TOF), ethyl acetate, and methanol extracts as well as essential oil (EO) obtained from tubers of *Cyperus rotundus* L. was assessed by "Ames assay", using *Salmonella* tester strains TA98 and TA100, and "SOS chromotest" using *Escherichia coli* PQ37 strain with and without an exogenous metabolic activation system (S9). None of the different extracts showed a mutagenic effect. Likewise, the antimutagenicity of the same extracts was tested using the "Ames test" and the "SOS chromotest". Our results showed that *C. rotundus* extracts have antimutagenic effects with *Salmonella typhimurium* TA98 and TA100 strains towards the mutagen Aflatoxin B1 (AFB1), as well as with *E. coli* PQ37 strain against AFB1 and nifuroxazide mutagens. A free radical scavenging test was used in order to explore the antioxidant capacity of the extracts obtained from the tubers of *C. rotundus*. TOF, ethyl acetate and methanol extracts showed an important free radical scavenging activity towards the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. These extracts showed IC50 values of respectively 5, 20 and 65 µg/ml. The beneficial effects of TOF, ethyl acetate, methanol and essential oil extracts of *C. rotundus* have been assessed by antioxidant and antimutagenic activities.

Key words: *Cyperus rotundus* tubers, essential oil, extracts, mutagenicity, antimutagenicity, free radical scavenging activity.

EINTRODUCTION

Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine. Investigation of traditionally used medicinal plants is thus valuable on two levels, firstly, as a source of potential chemotherapeutic drugs, and secondly, as a measure of safety for the continued use of medicinal plants. Plants and micro-organisms provide the pharmaceutical industry with some of the most important sources of components for the research of new medications.

In recent years there has been increasing interest in antimutagenesis (Calomme *et al*., 1996) and antioxidant activity (Vaya *et al.,* 2002) of plant origin compounds. Such compounds may be useful in preventing from cancer and other mutation-related diseases that can be pursued by avoiding exposures to recognised mutagens-carcinogens, by fortifying physiological defence mechanisms, or by favouring the intake of protective factors (De Flora, 1998). In fact according to Lee *et al.* (2003), free radical theory of ageing postulates that ageing is caused by excessive reaction of free radicals called reactive oxygen species (ROS). Therefore, researchers have made numerous efforts to find antioxidants. In view of several drawbacks of synthetic compounds for the human organism, examination of preparations of plant origin for this purpose has received more attention.

Cyperus rotundus (Cyperaceae) is a traditional herbal medicine used widely as analgesic, sedative, antispasmodic and to relieve diarrhoea (Thebtaranonth *et al*., 1995; Zhu *et al*., 1997). This plant, which grows naturally in tropical, subtropical and temperate regions, is widespread in North-East, Centre and South of Tunisia (Cuénod, 1954). The tuber part of *C. rotundus* is one of the oldest known medicinal plants used for the treatment of dysmenorrheal and menstrual irregularities (Bhattarai, 1993). Previous phytochemical studies showed that the major chemical components of this herb were essential oil, terpenoids, mono and sesquiterpenes (Ohira *et al*., 1998; Sonwa *et al*., 2001; Kilani *et al*., 2005a).

In this study, we reported mutagenic, antimutagenic and an antioxydant properties of several extracts from *C. rotundus* tubers.

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MATERIALS AND METHODS

Chemicals. Dimethylsulfoxide (DMSO), glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide phosphate (NADP), O-nitrophenyl-β-D-galactopyranoside (ONPG), P-nitrophenyl phosphate (PNPP), the positive mutagens Aflatoxin B1 (AFB1), nifuroxazide, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and α-tocopherol were purchased from Sigma-Aldrich, USA. Histidine, biotine, agar, bactotrypton, yeast extract and nutrient broth N° 2 were purchased from Difco, USA. Aroclor 1254 was purchased from Supelco, USA.

Plant material. Tuber parts of *C. rotundus* L., were harvested in the region of Monastir in the Centre of Tunisia, in June 2003. Botanical identification was carried out by Prof. Mohamed Chaieb (Department of Botany, Faculty of Sciences, University of Sfax, Tunisia), according to the flora of Tunisia (Cuénod, 1954). A voucher specimen (CP-06.03) has been deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy of Monastir, Tunisia.

Preparation of plant extracts. The tuber parts of *C. rotundus*, were dried at room temperature and reduced to coarse powder. The powdered was extracted with boiling water for 15 to 20 min. After filtration, the extracts were lyophilised (aqueous extract). The residues were dissolved in water. In order to obtain an extract enriched with TOF, the powder was macerated in water/acetone mixture (1:2 v/v), during 24 h with continuous stirring. The extract was filtered and the acetone was evaporated under low pressure in order to obtain an aqueous phase. Tannins were removed by precipitation with an excess of NaCl during 24 h at 5 °C, and the supernatant was recovered. This latter was extracted with ethyl acetate, concentrated and precipitated with an excess of chloroform. The precipitate was separated the TOF extract was yielded.

Ethyl acetate and methanol extracts were obtained by soxhlet extraction (6 h). The two types of extract, with different polarities were concentrated to dryness and the residue was kept at 4 °C. These two extracts were resuspended in DMSO. In the present study, four extracts were investigated.

The essential oil was extracted by steam distillation of the tubers part of *C. rotundus*, using the apparatus described in the 9th edition of the French Pharmacopoeia cited by Bruneton (1999) and analysed by gas chromatography-mass spectrometry (GC-MS).

Bacterial strains. *Escherichia coli* PQ37 and *Salmonella typhimurium* strains TA100 and TA98 were kindly provided by Prof. Felzen, UERJ, Brazil. The complete genotypes of *Escherichia coli* PQ37, as well as strain construction details are described by Quillardet and Hofnung (1985). Frozen permanent copies of the tester strain were prepared and stored at –80 °C.

Histidine-dependent strains, *S. typhimurium* TA100 and TA98 were maintained as described by Maron and Ames (1983). Strain TA98 allows detection of frameshift mutations. This strain contains the plasmide pKM 101. The products of the *mucAB* genes on this plasmid enhance SOS mutagenesis, thereby making strain TA98 more responsive to certain mutagens as AFB1. Strain TA100 is the most sensitive of all of the *S. typhimurium* tester strains (Zeiger *et al.,* 1985) particulary against AFB1. The genotypes of the test strains were checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr B* mutation) and the presence of the R factor. They were stored at –80 °C.

Preliminary phytochemical analysis. Plant materials were screened for the presence of tannins, flavonoids, coumarins and sterols by using the methods previously described by Tona *et al.* (1998) and Kilani *et al*. (2005b). Two milligrams of each extract was separately dissolved in 2 ml of the adequate solvent. The identification of major chemical groups was carried out by thin-layer chromatography (TLC) on silica gel 60 F254 Merck (layer thickness 0.25 mm) as follows: for flavonoids, TLC was developed in *n*-butanol/acetic acid/water 4:1:5 (top layer), spots were visualised with 1% aluminium chloride solution in methanol under UV (366 nm). Coumarins were detected under UV light (366 nm) thanks to their blue fluorescence which becomes intense after spraying 10% potassium hydroxide solution in ethanol. Steroids were identified with Libermann-Burchard, as reagent using *n*-hexane/CH_2Cl_2 1:9 as mobile phase. A range of colours are produced after heating sprayed plates for 10 min at 100 °C. The test for tannins was carried out with $FeCl_3$. Each class of tannins gave a specific coloration. The composition of the oil was investigated by gas chromatography (GC) and GC/MS (Kilani *et al*. 2005a).

Activation mixture. The S9 microsome fraction was prepared from rats treated with Aroclor 1254 (Maron and Ames, 1983). The composition of the activation mixture for the SOS chromotest was the following per 10 ml of S9 mix: 0.2 ml of salt solution (1.65 M KCl and 0.4 M $MgCl_2 \cdot 6H_2O$); 0.05 ml of 1 M G6P; 0.15 ml of 0.1 M NADP; 2.5 ml of 0.4 M Tris buffer, pH 7.4; 6.1 ml of Luria broth medium; 1 ml of S9 (Quillardet and Hofnung, 1985).

The components of S9 mix for the Ames test was 1 ml of salt solution; 0.25 ml of 1 M G6P; 2 ml of 0.1 M NADP; 25 ml of 0.2 M sodium phosphate buffer, pH 7.4; 7 ml of S9 and 14.75 ml H_2O. The S9 mix was prepared freshly for each assay (Maron and Ames, 1983).

Protein concentration of rat liver S9 was determined using protein BioRad assay (Bradford, 1976). It was found to be 12.3 mg/ml.

SOS chromotest assay. The procedure of SOS chromotest was performed according to Quillardet and Hofnung (1985). As a measure of anti-genotoxicity, the SOSIP (SOS-inducing potency) was calculated from the linear part of the induction factor dose-response curve. The induction factor (IF), was calculated as the ratio:

$$R_C / R_0$$

where R_C is equal to β-galactosidase (β-gal) activity / alcaline phosphatase (AP) activity determined for the test compound at concentration c, and R_0 is equal to β-gal activity / AP activity in the absence of test compound. The β-gal and AP activities were calculated according to the method recommended by Quillardet and Hofnung (1985). The assay was performed with and without an exogenous metabolic system, the S9 microsome fraction.

Anti-genotoxicity was expressed as percentage inhibition of the mutagenicity induced by AFB1 (10 µg/assay) or nifuroxazide (20 µg/assay), according to the formula:

Inhibition (%) = $[1-(IF_{assay} - IF_0 / IF_{mutagen} - IF_0)] \times 100$

where IF_0 is the induction factor of the negative control. Each dose was tested in triplicate.

Ames assay. The mutagenic effects of plant extracts were assessed according to the Ames test using *S. typhimurium* strains TA98 and TA100 (Maron and Ames, 1983). The assay was performed with and without, the S9 microsome fraction. The criteria for a positive response were a doubling for both TA98 and TA100 strains of the revertant solvent control value (Maron and Ames, 1983). Antimutagenic activity of the same extracts was assessed according to the Ames test. The mutagen used was AFB1 (10 µg/plate), which required S9 mix for metabolic activation. The inhibition rate of mutagenicity (%) was calculated relative to those in the control group with the mutagen by the following equation:

Inhibition rate (%) = [1-(number of revertants on test plates-number of spontaneous revertants) / (number of revertants on AFB1 control plates-number of spontaneous revertants)] x 100

Data were collected as mean ± standard deviation of three plates.

Radical scavenging activity. The free radical scavenging capacity of the compounds tested was determined with 1,1-diphenyl-2-picrylhydrazyl (DPPH). An aliquot of each compound tested at various concentrations (100, 30, 10, 3 or 1 µg/ml in ethanol) was mixed with 23.6 µg/ml of DPPH solution in ethanol. After incubation of the mixture for 30 min, the absorbance of the remaining DPPH was determined spectrophotometrically at 517 nm. The scavenging activities were expressed as a percentage of absorbance of the control DPPH solution (Vaya *et al*., 2002). The results are expressed as percentage activity from the following equation:

Activity (%)= $[(OD_{control} - OD_{sample}) / OD_{control}] \times 100$

Mean inhibiting concentrations IC_{50} (inhibitory concentration, 50%) were calculated by the use of Litchifield & Wilcoxon test (Galati *et al*., 2001).

RESULTS

Phytochemical study

The results of our assay on the crude extracts of *C. rotundus* tuber parts are shown in Table 1. TOF, ethyl acetate and methanol extracts showed important quantities of flavonoides, coumarins and tannins. In contrast, aqueous extract contained smaller quantities of tannins and coumarins. In the oil of *C. rotundus*, two monoterpene hydrocarbons (5.3%), one oxygenated monoterpene (0.3%), thirteen oxygenated sesquiterpenes (26.5%) and seventy components of sesquiterpene hydrocarbons (66.5%) were identified. The total amount of the sesquiterpenes fraction in the oil of *C. rotundus* (93%) was higher than the monoterpenes fraction (5.6%) (Kilani *et al.,* 2005 a).

Genotoxic and mutagenic activities of extracts

The results of mutagenicity with and without metabolic activation are reported in Table 2. Experiments realised with the tested extracts revealed no mutagenicity induction at the doses 500, 200 and 50 µg/plate in the *S. typhimurium* TA98 and TA100 assay system. The ability of the same extracts of *C. rotundus* to induce or not a mutagenic effect was also performed using the SOS assay system with *E. coli* PQ37 strain (Quillardet *et al.,* 1982; Kevekordes *et al.,* 1999). The experiments revealed no genotoxicity insofar as the induction factor (IF) isn't higher than 1.5 with the different tested concentration (Table 3). In fact, according to Kevekordes *et al.* (1999), compounds are classified as non-genotoxic if the IF remains < 1.5.

The absence of mutagenicity in different extracts and essential oil from *C. rotundus* tubers indicates that DNA does not seem to be relevant target for these extracts.

Antimutagenicity studies

The antimutagenicity testing of extracts from tubers of *C. rotundus* against the indirectly acting mutagen AFB1 (10 µg/assay) in the *S. typhimurium* TA98 and TA100 strains by the incorporation assay in the presence of metabolic activation, showed that they had a dose-related inhibitory effect on the mutagenic potential of this mutagen (Table 4).

When 50 µg/plate of respectively aqueous, TOF, ethyl acetate, methanol extracts and essential oil were added to the assay system, the number of revertants per plate for *S. typhimurium* TA100 decreased compared to those obtained in the absence of the extract and in the presence of the mutagen. The number of *S. typhimurium* TA98 revertants also showed a decrease. The inhibitory effect slightly increased when the concentration of the aqueous extract rises to 200 and 500 µg/plate with inhibition rates of 65.38 and 65.64% respectively with *S. typhimurium* TA100. Inhibitory increasing rates were ranged from 20.08 to 37.96% in *S. typhimurium* TA98 assay system.

Ethyl acetate extract showed an important antimutagenic activity against *S. typhimurium* TA100 and TA98. On

TABLE 1 - Phytochemical screening of extracts from tubers of *Cyperus rotundus*

Extracts	Yield (% w/w)	Tannins	Flavonoids	Coumarins
Aqueous extract	13.64	++	++	++
TOF extract	0.16	++++	++++	+++
Ethyl acetate extract	0.98	+++	++++	++
Methanol extract	15.10	+++	+++	+++

–: not detectable; ++: low quantities; +++: high quantities; ++++: very high quantities.

TABLE 2 - Evaluation of mutagenic activity of different extracts from *Cyperus rotundus* tubers by *Salmonella typhimurium* TA98 and TA100 assay systems, in the presence and absence of the exogenous metabolic activation system (S9)

Extracts	Dose (µg/plate)	His + revertants/plate			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Aqueous extract	50	22.5 ± 3.5	37 ± 3	99.5 ± 4.5	111 ± 3.5
	200	21 ± 2	33 ± 1	89 ± 3.5	183.5 ± 5.5
	500	20 ± 1	42 ± 2	95.5 ± 5.5	161.5 ± 4.5
TOF extract	50	21.5 ± 1.5	37 ± 2	84.5 ± 1.5	143.5 ± 0.5
	200	25.5 ± 2.5	30 ± 0	93.5 ± 5.5	117 ± 9.5
	500	26 ± 0	31 ± 1	99.5 ± 5.5	135 ± 10.5
Ethyl acetate extract	50	34.5 ± 8.5	20 ± 2	89 ± 1.5	87.5 ± 3.5
	200	33.5 ± 9.5	26.5 ± 3.5	88 ± 4	96.5 ± 2.5
	500	28 ± 3	22 ± 2	90 ± 3	104.5 ± 3.5
Methanol extract	50	32.5 ± 0.5	27 ± 2	109 ± 2.5	144 ± 4
	200	40 ± 5	34 ± 6	122 ± 2	148.5 ± 1.5
	500	42 ± 1	48 ± 11	121 ± 1	124.5 ± 10.5
Essential oil	50	34 ± 4	60 ± 4	86 ± 6	141 ± 6
	200	29 ± 2	58.5 ± 1.5	104 ± 2	133.5 ± 1.5
	500	25 ± 2	57 ± 7	102.5 ± 6.5	128 ± 8
Spontaneous revertants	-	21 ± 5	31.5 ± 0.5	99 ± 10	124.5 ± 8.5

TABLE 3 - Evaluation of mutagenic activity of different extracts from tubers of *Cyperus rotundus* by the SOS chromotest with *Escherichia coli* PQ37 strain in the absence and presence of the exogenous metabolic activation system (S9)

Extracts	Dose (µg/assay)	Without (S9)			With (S9)		
		β-gal (U)	AP (U)	IF	β-gal (U)	AP (U)	IF
NC	0	2.9 ± 0.01	12.57 ± 0.001	1	8.48 ± 0.005	11.33 ± 0.02	1
Aqueous extract	50	2.12 ± 0.01	17.10 ± 0.005	0.67	8.35 ± 0.009	11.5 ± 0.01	0.98
	200	2.72 ± 0.01	14.42 ± 0.01	0.81	8.64 ± 0.009	13 ± 0.02	0.89
	500	3.16 ± 0.02	13.72 ± 0.009	0.80	9.67 ± 0.01	14.09 ± 0.01	0.92
TOF extract	50	2.25 ± 0.005	13.72 ± 0.002	0.71	8.83 ± 0.008	20.03 ± 0.01	0.67
	200	1.15 ± 0.001	10.72 ± 0.01	0.46	4.72 ± 0.002	18.5 ± 0.01	0.39
	500	0.7 ± 0.01	9.15 ± 0.02	0.33	9.59 ± 0.005	20.93 ± 0.02	0.7
Ethyl acetate extract	50	9.84 ± 0.004	24.86 ± 0.01	1.16	8.56 ± 0.01	16.25 ± 0.01	0.81
	200	9.38 ± 0.005	25.2 ± 0.01	1.09	8.22 ± 0.01	16.03 ± 0.003	0.78
	500	7.54 ± 0.01	26.3 ± 0.03	0.84	7.5 ± 0.008	18.62 ± 0.01	0.62
Methanol extract	50	8.86 ± 0.01	26.66 ± 0.01	0.97	9.82 ± 0.01	17.68 ± 0.01	0.85
	200	10.72 ± 0.001	28.33 ± 0.005	1.11	8.38 ± 0.002	19.31 ± 0.008	0.66
	500	11.68 ± 0.007	29.26 ± 0.006	1.17	9.17 ± 0.004	22.25 ± 0.02	0.63
Essential oil	50	7.45 ± 0.004	23.26 ± 0.007	0.94	7.66 ± 0.005	16.06 ± 0.001	0.73
	200	05.9 ± 0.002	19.93 ± 0.007	0.87	7.53 ± 0.005	16.68 ± 0.01	0.69
	500	5.68 ± 0.006	19 ± 0.01	0.88	6.38 ± 0.002	15.71 ± 0.01	0.62

β-gal (U): β-galactosidase units; AP (U): alkaline phosphatase units; IF: induction factor; NC: negative control.

TABLE 4 - Effect of extracts and essential oil on mutagenicity induced by AFB1 (10 μg/plate) in *Salmonella thyphimurium* TA100 and TA98 assay systems in the presence of S9

Extracts	Dose (μg/plate)	TA98		TA100	
		No. revertants/plate	Inhibition of mutagenesis (%)	No. revertants/plate	Inhibition of mutagenesis (%)
Spontaneous	-	27 ± 1	-	115.5 ± 12.5	-
AFB1	10	729 ± 20	-	310.5 ± 13.5	-
Aqueous extract	50	654.5 ± 20	10.61	185 ± 14	64.35
	200	588 ± 2	20.08	183 ± 10	65.38
	500	462.5 ± 30	37.96	182.5 ± 0.5	65.64
TOF extract	50	275.5 ± 10.5	64.6	165.5 ± 0.5	74.35
	200	155 ± 11	81.76	154 ± 0	80.25
	500	122 ± 10	86.46	145 ± 14	84.87
Ethyl acetate extract	50	266 ± 5	65.95	166.5 ± 2.5	73.84
	200	153 ± 20	82.05	158.5 ± 10.5	77.94
	500	133.5 ± 1.5	84.82	148 ± 10	83.33
Methanol extract	50	405 ± 30	46.15	193 ± 6	60.25
	200	306.5 ± 18.5	60.18	185 ± 7	64.35
	500	280 ± 11	63.96	177 ± 7	68.46
Essential oil	50	129 ± 18	85.47	149.5 ± 0.5	82.56
	200	156 ± 20	81.62	175 ± 3	69.48
	500	215 ± 15	73.21	183 ± 10	65.38

AFB1: positive control.

TABLE 5 - Effect of extracts on genotoxicity induced by nifuroxazide (20 μg/assay) without exogenous metabolic activation system (S9)

Extracts	Dose (μg/assay)	β-gal (U)	AP (U)	IF	Inhibition of mutagenesis (%)
Nifuroxazide	20	11.89 ± 0.004	14.21 ± 0.01	7.6	-
NC	0	2.26 ± 0.0005	19.5 ± 0.01	1	-
Aqueous extract	200	7.95 ± 0.007	13.46 ± 0.01	5.36	33.93
	500	7.65 ± 0.005	14.53 ± 0.004	2.75	42.57
TOF extract	10	8.59 ± 0.005	14.17 ± 0.002	5.5	31.7
	50	4.09 ± 0.007	9.46 ± 0.01	3.93	61.44
	200	1.9 ± 0.005	8.71 ± 0.02	1.98	85.15
Ethyl acetate extract	50	8.93 ± 0.01	11.59 ± 0.02	5.67	29.24
	200	6.1 ± 0.03	8.65 ± 0.003	4.04	53.93
	500	3.2 ± 0.005	12.46 ± 0.01	2.84	72.12
Methanol extract	50	8.18 ± 0.004	12.75 ± 0.01	4.93	40.45
	200	7.76 ± 0.09	12.71 ± 0.01	4.69	44.09
	500	7.15 ± 0.03	12.06 ± 0.01	4.55	46.21
Essential oil	1	8.25 ± 0.005	12.69 ± 0.003	5	35.69
	5	6.01 ± 0.06	12.56 ± 0.01	3.68	56.91
	10	5.78 ± 0.01	12.34 ± 0.01	3.6	58.19

β-gal (U): β-galactosidase units; AP (U): alkaline phosphatase units; IF: induction factor; nifuroxazide: positive control; NC: negative control.

TABLE 6 - Effect of extracts on genotoxicity induced by AFB1 (10 µg/assay) with exogenous metabolic activation system (S9).

Extracts	Dose (µg/assay)	β-gal (U)	AP (U)	IF	Inhibition of mutagenesis (%)
AFB1	10	37.45 ± 0.004	7.16 ± 0.01	10.25	-
NC	0	3.96 ± 0.01	7.66 ± 0.02	1	-
Aqueous extract	50	33.81 ± 0.002	6.64 ± 0.01	9.98	2.84
	200	27.36 ± 0.001	5.99 ± 0.01	8.95	13.97
	500	22.72 ± 0	5.25 ± 0.002	8.48	19.04
TOF extract	10	18 ± 0.01	4.62 ± 0.007	7.63	28.31
	50	10.83 ± 0.006	4.96 ± 0.003	4.28	64.52
	200	6.25 ± 0.003	4.71 ± 0.01	2.60	82.68
Ethyl acetate extract	50	7.97 ± 0.001	4.35 ± 0	3.59	72
	200	10 ± 0	5.58 ± 0.01	3.51	72.86
	500	7.05 ± 0.004	4.42 ± 0.01	3.12	77
Methanol extract	50	20.36 ± 0.01	4.61 ± 0.004	8.65	17.29
	200	18.52 ± 0.03	4.42 ± 0.01	8.21	22.05
	500	10.36 ± 0.006	3.91 ± 0.008	5.19	54.72
Essential oil	1	10.83 ± 0.01	5.18 ± 0.01	3.8	24.49
	5	10.5 ± 0.004	6.36 ± 0.02	3	46.09
	10	9.41 ± 0.01	7.06 ± 0.01	2.42	61.72

β-gal (U): β-galactosidase units; AP (U): alkaline phosphatase units; IF: induction factor; AFB1: positive control; NC: negative control.

the other hand, methanol extract showed similar antimutagenic activity against strains TA100 and TA98.

In turn, the TOF extract obtained from *C. rotundus* showed an important inhibitory effect on the mutagenicity induced by AFB1 with *S. typhimurium* TA100 and TA98 assay system.

As shown in Tables 5 and 6, all extracts from *C. rotundus* were effective in reducing the IF induced by AFB1 (10 µg per assay with S9: indirect-acting genotoxic), as well as by nifuroxazide (20 µg per assay without S9: direct-acting genotoxic).

TOF (at a dose of 200 µg/assay) and ethyl acetate extracts (at a dose of 500 µg/assay) were more effectively antigenotoxic than the aqueous and methanol extracts. TOF, ethyl acetate, aqueous extract and methanol extracts significantly decreased the SOSIP of AFB1, by respectively 82.68, 77, 19.04 and 54.72%. At the same concentration of these extracts, the SOSIP of nifuroxazide decreased about respectively 85.15, 72.12, 42.57 and 46.21%.

Essential oil of *C. rotundus* was also effective in reducing nifuroxazide and AFB1 induced mutagenicity in *E. coli PQ37* strain. This effect was more pronounced at lower doses. The SOSIP of nifuroxazide decreased by 35.69, 56.91 and 58.19% when respectively 1, 5 and 10 µg/assay of EO were added to the *E. coli* PQ37 assay system. Whereas, the SOSIP of AFB1 decreased by 61.72, 46.09 and 24.49% when respectively 10, 5 and 1 µg/assay of the same extract were added to the assay system.

Scavenging activity on DPPH radical

DPPH is a molecule containing a stable free radical. In the presence of an electron-donating anti-oxidant, the purple colour typical of the free DPPH radical diminishes in intensity, a change that can be followed spectrophotometrically at 517 nm. This simple test provides information on the ability of a compound to donate an electron, on the number of electrons a given molecule can donate, and on the mechanism of anti-oxidant action.

The radical-scavenging activities of the extracts, measured as decolourising activity following the trapping of the unpaired electron of DPPH, are shown in Table 7. TOF and ethyl acetate extracts were very potent radical scavenger with IC_{50} values of respectively 5 and 20 µg/ml.

The methanol extract showed scavenging activity with a percentage decrease versus the absorbance of the DPPH standard solution of 57.52% at a concentration of 100 µg/ml and IC_{50} value of 65 µg/ml.

EO from C. *rotundus* showed a weak scavenging activity.

DISCUSSION

Antimutagenic properties elicited by *C. rotundus* have a full range of perspective applications in human healthcare. Herbals remedies and phytotherapic drugs containing active principles are currently developed to protect against electrophile (e.g. free radical) attack to DNA and its widespread outcomes such as ageing and cancer. Even for populations which use herbs traditionally, encouraging the use of species with chemopreventive actions could be helpful as part of life expectancy improvement strategies: costs are significantly low, herbs have usually little or no toxicity during long-term oral administration and are available at large scale.

TOF and ethyl acetate extracts from *C. rotundus* exhibit a strong binding activity towards the free radical DPPH. Whereas methanol extract and EO exhibit a less strong activity towards the same radical.

TABLE 7 - Antioxidant activities on scavenging the DPPH free radical of extracts from tubers of *Cyperus rotundus*

Extracts	Concentration (µg/ml)	Inhibition (%)[a]	IC_{50} (µg/ml)
α-Tocopherol	1	30 ± 2.1	3.1
	3	50 ± 1.3	
	10	97.3 ± 1.8	
	30	98 ± 1.3	
	100	98.7 ± 2.2	
Aqueofus extract	1	0	> 100
	3	0	
	10	0	
	30	0	
	100	18.58 ± 2	
TOF extract	1	7.52 ± 1	5
	3	62.38 ± 0.9	
	10	64.15 ± 0.6	
	30	69.02 ± 2.1	
	100	77.43 ± 1.5	
Ethyl acetate extract	1	4.86 ± 1.8	20
	3	14.15 ± 2.5	
	10	22.56 ± 1.2	
	30	63.71 ± 1.3	
	100	74.77 ± 1.2	
Methanol extract	1	0.44 ± 2.1	65
	3	8.40 ± 0.8	
	10	11.65 ± 1	
	30	23.45 ± 1.2	
	100	57.52 ± 1.1	
Essential oil	1	0	> 100
	3	0	
	10	0	
	30	0	
	100	40.34 ± 1.1	

[a]: Inhibition of absorbance at 517 nm relative to that of standard DPPH solution; α-tocopherol: positive control.

In both Ames and SOS chromotest, TOF, ethyl acetate extracts and EO of *C. rotundus* tubers strongly inhibited the genotoxicity of AFB1. The weak antimutagenic activity of essential oil when large excess of the extract is added could be explained by inhibition of the penetration through the cell at high doses, of molecules which are implied in the mutagenic inhibitory effect towards AFB1. The inhibitory effect of the tested extracts on the genotoxicity of AFB1 may be attributed to flavonoids (Calomme *et al.,* 1996; Shon *et al.,* 2004), coumarins (Lee *et al.,* 2000), tannins (Schimmer *et al.,* 1992) for extracts, and terpenes, mono and sesquiterpenes for EO (Ohira *et al.,* 1998; Sonwa *et al.,* 2001). This is supported by the weak antigenotoxic activities obtained with aqueous extract reported in this study, in sofar as this extract contains low quantities of tannins, flavonoids and coumarins, as shown by chemical analysis. We cannot however, exclude the possibility that other compounds with antigenotoxic properties participate in the inhibitory effect. The results of our experiments confirm the known antioxidant activities of flavonoids (Vaya *et al.,* 2002; Park *et al.,* 2004) and tannins (Yokozozawa *et al.,* 1998). Flavonoids are the most likely candidates among the compounds known to be present in the TOF and ethyl acetate extracts, for providing the antimutagenic effect and preventing oxidative lesions (Edenharder and Grunhage, 2003).

The TOF, ethyl acetate extracts and EO showed significant antigenotoxic activity by the SOS chromotest assay against nifuroxazide and AFB1. This suggests that these extracts inhibit microsomal enzyme activation or that they directly protect DNA strands from the electrophilic metabolites of the mutagens. However, the inhibition of genotoxicity and mutagenesis is often complex, acting through multiple mechanisms (Kada *et al.,* 1993). In fact compounds and complex mixtures with antimutagenic activity have different modes of action and act in parallel at different levels; as inhibitors, they may prevent the formation of mutagens; as blocking agents, they can prevent the biotransformation of pre-mutagens into reactive metabolites by inhibiting metabolic activation induced by the P450 (as in cytochrome P450) and/or mono-oxygenase system. This inhibitory effect is known to play a role in the antimutagenic activity of some plant extracts (Zani *et al.,* 1993) against AFB1 and, we suspect also, in the case of *C. rotundus* extracts. They can also prevent the biotransformation of pre-mutagens by scavenging reactive molecules (Krul *et al.,* 2001). In this case extract may reduce mutagenic effect by blocking the oxidation process induced by the control mutagens; as suppressing agents they may modulate intracellular processes, which are involved in DNA repair damages mechanisms. These damages may be induced by free radicals.

Essential oil and methanol extract showed low antioxidant activity but very strong antimutagenic activity. Thus, in our study we could not find a direct correlation between the antioxidant capacity and antimutagenic activity of these two extracts, even though the antioxidants are probable antimutagens. This may be due to variation in the quality and quantity of bioactive compounds present in these latter extracts

In summary, *C. rotundus* tuber extracts and their components could be suitable antigenotoxic, antimutagenic and perhaps anticarcinogenic agents, but further studies to fractionate the active extracts, to identify the active compounds and to determine the exact mechanism of action, are required.

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