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STUDY OF THE INHIBITORY ACTIVITY OF THE CELLULAR GROWTH OF VETIVER'S AQUEUOUS AND METHANOL-BASED EXTRACTS

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ABSTRACT

In order to evaluate the antimitotic activity of *Vetiveria zizanioides*, a study was carried out on its plant cells using the *Lepidium sativum* L bioassay. We conducted the optimization of several parameters related to different modes of extraction. The root extract obtained by decoction through methanol exhibits the best activity with 72.43% inhibition, followed by leaf extract obtained by decoction through distilled water with 63.8% inhibition. However, the different concentrations of the Vetiver grass essential oil provide low percentage inhibition, below 40%.

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INTRODUCTION

Cancer treatment has known, for a long time now, numerous breakthroughs thanks to the successive introduction of new antimitotic products in the therapeutic arsenal.

Medicinal plants form non-negligible natural resources at the foundation of these medicines. [a] [b] [c] [d] [e]

The Vetiver is a plant known since the beginning of the last century. There are 12 known varieties of Vetiver grass, the most important being the *Vetiver zizanoides*. The first uses of this plant concerned cosmetics through the extraction of its essence, as well as health with the infusion based upon it, and also ecology for its ability to keep lands moisture and prevent the pollution of natural resources.

This plant was still unheard of in Morocco, and we wanted to study one of its particularly unknown aspects, namely its cytotoxicity. For this purpose, we used a plant test (phytotest, Lépidium sativum) that consists in measuring the inhibition of cellular growth.

Experimental

Biological material:

The *Vetiver*, also called *Chrysopogon*, is a grass belonging to the *Poaceae* family [f]. The *Vetiveria zizanioides*, originally from Asia, mainly grows in India [g]. This species has two genotypes that are used for lands and water conservation, as well as for lands stabilization in India: the seed wild-type genotype of North India and the unproductive genotype of South India [h]. The unproductive genotype is the main form of *Vetiver* used in the production of essential oil intended for cosmetic products, and spread worldwide for lands conservation and stabilization [i]. The plant taxonomy was established as follows:

Phylum	**Spermatophyte**
Subphylum	Angiosperms
Class	Monocotyledons
Subclass	Commelinids
Order	Cyperales
Family	Gramineae (Poaceae)
Genus	Chrysopogon
Species	*Vetiveria zizanioides L. Crysopogon zizanioides R*

Extractions:

Extraction through Soxhlet:

6 g of the dry plant were extracted to end of stock, using two solvents with growing polarity (distilled water (DW) and methanol), in hot conditions, through a Soxhlet extractor. Residues obtained after evaporation were tested.

Decoction:

6 g of the dry plant were extracted by decoction during 20 minutes, using two solvents with growing polarity (DW and methanol). Residues obtained after evaporation were tested.

Extraction of the essential oil:

The extraction of Vetiveria zizanioides (L.) essential oil was made through hydro-distillation using a Clevenger-type device. The process has been realized during 12 hours.

Biological test:

Evaluation methods of antimitotic effects through the study of mitosis on vegetal cells are numerous, well developed in vitro and in vivo, as shown in multiple scientific papers and especially those of TRUHAUT and DEYSSON [j] - [k], dealing with onion bulbs (*Allium cepa* L) or wheat caryopses (*Triticum vulgare*, [l]).

GAGIU et al. [m], used a new vegetable substrate, the *Lépidium sativum* L, that lead them to obtain a test presenting many benefits compared to the previous ones.

A great many authors proposed tests enabling to quickly and simply evaluate potential cytostatic activity of new compounds located on different parts of plants. These tests concern a global action on vegetal growth and cannot, in any circumstances, determine the mechanism of action of antimitotic molecules. [n] [o] [p] [q] [r] [s]

We used a biological test based on the measurement of the length of the *Lépidium sativum* L. sprout seed rootlet, seed put in an environment that contains the substance to test. We evaluated the percentage of growth inhibition by comparing it with a control sample.

Protocol:

To *Lepidium sativum* L seeds, sprouting for 24h, we add the substance to test in a specific concentration, solubilized either in distilled water or in a aqueous colloidal solution of methylcellulose 1%. The extracts to test are obtained from the different organs, through Soxhlet extraction and decoction.

We realize jointly for every series of experience, on the one hand a control sample that only contains distilled water, and on the other hand a reference sample that contains colchicine at a given concentration. [s]

RESULTS AND DISCUSSION

We mobilized an analysis of variance (ANOVA) with two factors, which showed there is a significant relation ($p < 0.05$) between the percentage of inhibition (I%) and the concentration of the tested extracts. But the behavior of the percentage of inhibition does not seem to change with the extraction method. (Figure 1 and Table 1).

We notice that the percentages of growth inhibition of the different tested extracts have exceeded 50%:

- At the concentration of 8 mg/ml
- For the ADL with 4 mg/ml

We use Tukey's range test (honest significant difference HSD) to discern means that are significantly different from each other. Thus, we can assure that the percentages of inhibition of the extracts obtained through decoction (whether with DW or methanol) are significantly higher ($p < 0.05$) than those obtained through Soxhlet extraction (Figure 2). A bilateral Dunnet comparison with a control sample (PCC), realized with a confidence interval of 95%, showed a significant difference between ways of extraction.

Table 1: Percentage of inhibition of different tested extracts of *Vetiveria zizanioides* (L.), facing Colchicine at different concentrations.

Tested extract	**Percentage of inhibition (I%)**			
	Concentration (mg/ml)			
	8	**4**	**2**	**Average**
ADL (Aqueous decoction, leaves)	79,35±0,41	55,93±0,11	25,59±0,61	53,62
ADR (Aqueous decoction, roots)	76,58±1,21	41,07±0,79	18,74±0,45	45,49
MDL (Methanol-based decoction, leaves)	77,62±0,31	33,72±0,51	13,65±0,58	41,66
MDR (Methanol-based decoction, roots)	79,28±0,76	46,66±0,61	20,64±0,44	48,86
SAL (Soxhlet aqueous, leaves)	57,05±0,91	24,68±0,42	15,06±0,91	32,26
SML (Soxhlet methanol, leaves)	71,70±0,57	36,03±0,32	14,25±0,26	40,66
Average	73,6	39,68	17,99	
	Concentration (mg/ml)			
	1	0,5	0,25	
PCC (Positive control : Colchicine)	83,58±1,21	60,38±0,35	27,45±0,51	57,14

The leaves extract obtained through water decoction (ADL) at 8mg/ml, presents the best activity with 79.35 % inhibition, followed by the root extract obtained through methanol decoction (MDR) at the same concentration, with 79.28% inhibition.

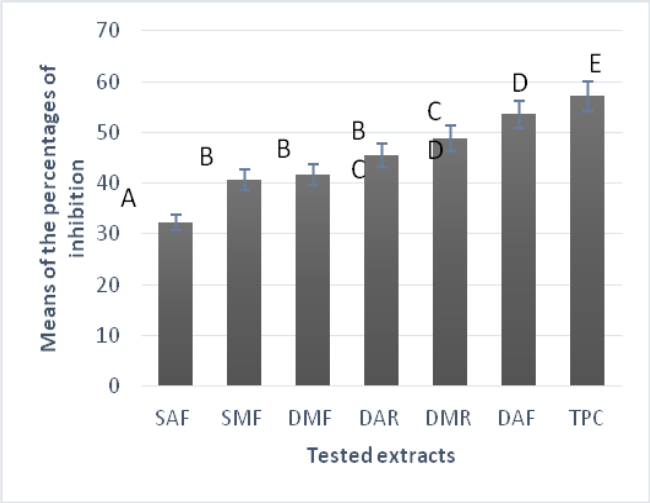

Figure 2: Average percentage of inhibition of the different tested extracts. The differences between these means according to Tukey's HSD are formulated with letters in growing order.

The germination of watercress grains treated by different concentrations of each extract was very low after 24h. No cytotoxic effect was found after the treatments, which implies that the extracts only inhibit grains germination, but do not damage them permanently.

But the evaluation of the inhibition growth activity of garden cress, for essential oil and herbal distillate, showed a low activity, inferior to 40%, for the different concentrations tested. (Table 2)

Table 2: Percentage of inhibition, with different concentrations of essential oil and herbal distillate of *Vetiveria zizanioides* (L.), *: total absence of activity.

Tested extract	Percentage of inhibition (I%)						
	Concentration (ml/ml)						
	0,1	0,04	0,02	0,01	0,005	0,003	0,002
Essential oil	36,59±0,61	32,11±0,98	28,01±0,24	23,09±0,47	16,87±0,66	12,37±0,14	6,74±0,74
Herbal distillate	22,09±0,85	10,89±0,12	9,72±0,47	3,62±0,24	0,42±0,49	*	*

CONCLUSIONS

We can conclude from our results that the inhibitory activity of the cellular growth of the Vetiveria zizanioides (L.) is due to one or more active compounds that present very high inhibition rates, the most significant inhibition rates being obtained through decoction with distilled water. Determining these compounds, identifying them and their allocation throughout the different organs of the plant remains to be done, in order to verify the obtained results within animal material (*in vitro or in vivo*).

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