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Cytotoxic and genotoxic activity of *Achillea millefolium* aqueous extracts

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Abstract: The yarrow, *Achillea millefoilum* L. is an important species of Asteraceae family with common utilization in folk medicine of many countries. The aim of this study was to investigate the cytotoxic and genotoxic effects of aqueous extracts from yarrow leaves on *Lactuca sativa* (lettuce) root tip meristem cells by cytogenetic studies, since studies of this nature do not exist for the yarrow extracts. For this, lettuce seeds were treated for 72 h with different concentrations of yarrow aqueous extracts (5, 10, 20 and 30 mg/mL). The percentage of germination, root development and cellular behavior were analyzed and the results showed that the highest concentration of aqueous extracts reduced the mitotic index (MI), the seed germination and the root development of *L. sativa*. More yet, the extracts also induced chromosome aberrations (CA) and cellular death in the roots cells of *L. sativa* indicating precaution in the therapeutic use of *A. millefolium* and reinforcing the utilization of *L. sativa* in the screening of cytogenotoxic substances once that this species showed a good sensibility to the extract application.

Introduction

The use of medicinal plants is probably as old as humankind itself. More than 150000 plant species have been studied, and many of them contain therapeutic substances. These substances can be extracted and used in the preparation of drugs, or the plant itself can be used directly as a medication, a practice that is particularly popular in developing countries. However, medicinal plants in conjunct with no medicinal plants, in general synthesize toxic substances, wich in nature act as defense against infections, insects and herbivores, being necessary caution in yours consumption for this (Negrelle & Gomes, 2007).

For man, among the damages caused by the toxic compounds presents in medicinal plants, genotoxic and mutagenic effects have shown to be worrying, due to its capacity to induce genetic damage, wich can lead several health problems and also affect future generations, since these alterations can be inheritable. Hence, the necessity to identify compounds that react with DNA in order to assure the medicinal compounds quality has led to the development of several genotoxicity and mutagenicity assays in a wide range of organisms (Çelik & Aslantürk, 2006, 2007; Negrelle & Gomes, 2007; Pugliesi et al., 2007; Lubini et al., 2008).

Test systems can be divided into groups based on the biological system employed and their genetic endpoint detected. Bioassays with prokaryotes enable the detection of agents that induce gene mutation and primary DNA damages. On the other hand, analyses with eukaryotes enable the detection of a great damage extent, varying from gene mutations to chromosome damages and aneuploidies and polyploidies (Leme & Marin-Morales, 2009).

Cytogenetic assays have been widely used in genotoxicity assessment to test compounds under both *in vitro* and *in vivo* conditions and alterations on the mitotic index (MI), micronuclei formation (MN), nuclear abnormalities (NA) and chromosome aberrations (CA) are important cytogenetic endpoints that are routinely used in citotoxicity and genotoxicity evaluation (SanSebastian et al., 1990; Krishna et al., 1991; Chacon et al., 2002; Ferreira et al., 2003; Zanoni et al., 2005; Kuras et al., 2006; Juchimiuk et al., 2007; Pugliesi et al., 2007; Campos et al., 2008; Leme & Marin-Morales, 2009).

Regarding plant bioassays, *Allium cepa, Zea mays, Vicia faba, Tradescantia, Nicotiana tabacum, Crepis capillaries* and *Hordeum vulgaris* have been the most common species used for cytogenotoxicity evaluation. This can be explained mainly by the great number of seeds produced by these species, the easy handling and by the great contact surface proportioned by bulbs and/or seeds in the aqueous extracts administrated. In addition, these species also show high sensibility to toxic compounds and they have large chromosomes, increasing their application for cytogenetic studies (Singh & Das, 2002; Sobita & Bhagirath, 2005; Campos et al.,

2008; Lubini et al., 2008).

According to Campos et al. (2008), the high sensibility and the great size of the *Lactuca sativa* (lettuce) chromosomes also make this plant very useful for cytogenetic analysis, nevertheless, this species have been used principally for detection of allelochemicals in experiments associated to the exploration of bioherbicides, being the root development the principal approach utilized in this study system, with little works making use of cytogenetic of this species in cytogenotoxicity screening studies.

Achillea millefolium L., Asteraceae, a plant popularly known as "yarrow", is native from Europe, although perfectly adapted to Brazil and other countries of South America, China and India. Popular indications of this medicinal species include treatment of wounds, hemorrhages, headaches, inflammation, pain, spasmodic diseases, flatulence, and dyspepsia (Correia, 1974; Chandler et al., 1982; Blumenthal et al., 2000; Lorenzi & Matos, 2002), being the species included in the Pharmacopoeias of innumerous countries as Brazil, Germany, Czech Republic, France and Switzerland with innumerous works showing its phytochemical and pharmacological application (Mitich, 1990; Bradley, 1992; Alonso, 1998; Blumenthal et al., 2000).

About its phytochemistry, studies have identified the compounds: achilleine (Miller & Chow, 1954), azulene and chamazulene (Haggag et al., 1975; Kokkalou et al., 1992; Kubelka et al., 1999), 1,8 cineole (Kokkalou et al., 1992), and flavonoids such as apigenin and luteolin (Valant Vetschera & Wollenweber, 1988; Guédon et al., 1993) in the aerial parts, but only a few studies correlated these substances with pharmacological activities or toxicity. Health hazards associated with long-term exposure to yarrow's extracts are not well established being this approach a very important and necessary field of study.

Despite the Food and Drug Administration has classified the species as non-poisonous and has approved its utilization in alcoholic drinks (Duke, 1987) some toxic effects had been reported after its use by humans and in animal experiments. The effects in human, for example include, contact dermatitis (Hausen et al., 1991). On the other hand, in others animals the yarrow's preparations reduced fetal weight when given to pregnant rats (Boswell-Ruys et al., 2003) and had antispermatogenic effects in mice (Montanari et al., 1998).

Considering the potential therapeutic use of *A. millefolium* extract, and the absence of data about its mutagenicity by cytogenetic bioassays, the aim of the present work was describes the effects of yarrow's leaves aqueous extracts on seed germination, root growing, chromosome structure and cell cycle behavior of *Lactuca sativa.*

Materials and Methods

Plant material and extracts preparation

Fresh leaves of *Achillea millefolium* L., Asteraceae, were collected at the Botanical Experimental Area of Universidade Federal de Juiz de Fora, Juiz de For a-MG, Brazil (21°13'33"S and 43°46'26"W). The plant was identified by Dr. Fátima Regina Gonçalves Salimena, at the Botany Department of UFJF and a sample was deposited at the CESJ Herbarium of the UFJF (voucher specimen number 52.627). Four aqueous extracts concentrations were prepared (Yar_1 = 5, Yar_2 = 10, Yar_3 = 20 and Yar_4 = 30g, respectively, in 1000 mL of distilled water). After 24 h, at room temperature, the extracts were filtered in filter paper before the application in the seeds bioassays.

Germination and root growth

The treatments were arranged in a completely random design with four repetitions at 28 °C. Each repetition corresponding to sixty seeds of lettuce placed in a Petri dish. As a control, we used lettuce seeds exposed to distilled water. The germination percentage and root growth were evaluated after 12, 24, 36, 48, 60 and 72 h of exposition time. The germination percentage was obtained by the counting of seeds with apparent roots in each one of the valuation time as described previously. The root growth were obtained by the use of one caliper, the measure were obtained in each one of the evaluation time.

Cytogenetics studies

After 72 h, ten roots were collected of each repetition (forty roots for treatment). These roots were fixed in a fresh cold methanol/glacial acetic acid solution 3:1 (v/v) during 24 h. Following fixation, the roots were submitted to an enzymatic maceration (Pectinex Novo Nordisk™) at 34 °C for 1:45 h. Subsequently, the roots were hydrolyzed in 5N HCl for 11 min. By air dry technique (Carvalho & Saraiva, 1993), five slides were prepared for each repetition (twenty slides for treatment) and stained with Giemsa 10% during 3 min. Mitotic index (MI) was determined for each treatment and the presence of chromosomes abnormalities (CA) was also evaluated.

Statistical analyses

The percentage of germinated seeds, root size, mitotic index and percentage of chromosome aberrations were evaluated. The data were submitted to one-way analysis of variance (ANOVA) and comparisons among the average of each treatment with the average of control were carried out using Tukey test ($p< 0.05$).

Results

In the present study, the mean percentage of germinated seeds decreased with the increment of the concentrations of yarrow extracts administered. The Yar_2 (10 mg/mL) treatments at times of 48, 60, and 72 h and Yar_3 (20 mg/mL) at times of 60, 72 h, showed significant difference when compared with the control, while the treatment Yar_4 inhibited completely the germination of lettuce. The treatment Yar_1 showed a similar behavior with the seeds germination in the control (Table 1).

The extract concentrations also showed a considerable influence in the time of seed germination. We observed that seeds treated with higher concentrations of yarrow extracts (Yar_2 and Yar_3) initiated the germination only after 48 and 60h respectively (Table 1).

In a general way, similar behavior was observed on root growth, with the treatments Yar_2 and Yar_3 reducing significantly (p<0.05) the root size when the average was compared with the control (Table 2). The higher concentrations of the extracts also show cytotoxic effects on root cells of lettuce once that the mitotic index decreased significantly when compared to the control in the Yar_2 and Yar_3 treatment (Table 3).

Together with the mitotic index, the cytological evaluation also revealed the occurrence of chromosome aberrations in the seeds treated with the highest concentrations of the extracts, more precisely with Yar_3 (20 mg/L) application. It was observed a great percentage of lagging migration of the chromosomes, chromosome bridges, chromosome breaks, chromosome stickiness, polar deviation and micronucleus (Table 4; Figure 1).

Additionally to these observations, on Yar_2 and Yar_3 treatments, cells with nuclear condensation and some nucleus with communications among them (nuclear bridge) were observed. Again, Yar_2 and Yar_3 treatments showed significant differences (p<0.05) when compared with the control (Table 5, Figure 1).

Table 1. Percentage mean of germination of lettuce seeds after 12, 24, 36, 48, 60 and 72 h of exposure to yarrow aqueous extracts.

Treatments	Exposure (h)					
	12	24	36	48	60	72
Control	-	78.31±1.33	87.12±1.02	91.22±1.16	94.01±1.21	95.11±1.15
Yar_1	-	75.93±1.02	85.03±1.12	88.90±1.65	93.06±1.22	94.21±1.31
Yar_2	-	-	-	57.69±1.69*	68.02±1.25*	82.31±1.21*
Yar_3	-	-	-	-	51.23±1.02*	58.24±1.24*
Yar_4	-	-	-	-	-	-

Yar_1: Yarrow aqueous extract (5 mg/mL), Yar_2: Yarrow aqueous extract (10 mg/mL), Yar_3: Yarrow aqueous extract (20 mg/mL), Yar_4: Yarrow aqueous extract (30 mg/mL). *Significantly different from the control (p< 0.05) (Tukey test).

Table 2. Mean size (cm) of lettuce roots during 72 h of exposure to Yarrow aqueous extracts.

Treatments	Exposure (h)					
	12	24	36	48	60	72
Control	-	0.71±0.04	1.11±0.06	1.62±0.08	2.60±0.08	3.09±0.12
Yar_1	-	0.68±0.06	0.98±0.09	1.57±0.05	2.51±0.09	3.01±0.13
Yar_2	-	-	-	1.02±0.07*	1.29±0.25*	1.87±0.31*
Yar_3	-	-	-	-	0.86±0.05*	1.05±0.04*
Yar_4	-	-	-	-	-	-

Yar_1: Yarrow aqueous extract (5 mg/mL), Yar_2: Yarrow aqueous extract (10 mg/mL), Yar_3: Yarrow aqueous extract (20 mg/mL), Yar_4: Yarrow aqueous extract (30 mg/mL). *Significantly different from the control (p< 0.05) (Tukey test).

Table 3. Mean of cells evaluated, dividing cells and mitotic index from lettuce roots cells after 72 h of exposure to Yarrow aqueous extracts.

Concentration	Mean of cells observed	Mean number of dividing cells	Mean of Mitotic Index
Control	18,721	1,350	7.21±1.51
Yar_1	16,321	1,110	6.89±1.38
Yar_2	17,229	345	2.02±1.12*
Yar_3	15,456	235	1.52±0.23*
Yar_4	-	-	-

Yar_1: Yarrow aqueous extract (5 mg/mL), Yar_2: Yarrow aqueous extract (10 mg/mL), Yar_3: Yarrow aqueous extract (20 mg/mL), Yar_4: Yarrow aqueous extract (30 mg/mL). *Significantly different from the control (p<0.05) (Tukey test).

Table 4. Mean percentage of chromosomes aberrations in meristematic cells of lettuce after 72h of exposure to Yarrow aqueous extracts.

Concentration	Chromosome aberrations					
	Lm	Brks	Stk	Sa	ABr	Mcn
Control	0.26±0.08	0.30±0.09	0.41±0.44	0.16± 0.05	0.71±0.08	0.12±0.04
Yar_1	0.22±0.04	0.34±0.10	0.51±0.22	0.30± 0.12	0.88±0.04	0.15±0.05
Yar_2	0.30±0.05	0.91±0.14*	0.61±0.31	0.22± 0.04	15.97±1.41*	0.13±0.03
Yar_3	12.26±2.87*	1.12±0.17*	3.27±1.21*	14.76± 2.97*	34.12±2.28*	1.57±0.06*
Yar_4	-	-	-	-	-	-

Yar_1: Yarrow aqueous extract (5 mg/mL); Yar_2: Yarrow aqueous extract (10 mg/mL); Yar_3: Yarrow aqueous extract (20 mg/mL); Yar_4: Yarrow aqueous extract (30 mg/mL). *Significantly different from the control ($p< 0.05$) (Tukey test). Lm: lagging migration and chromosome loss; Brks: breaks; Stk: stickiness; Sa: spindle alterations; ABr: anaphases with bridge; Mcn: micronuclei. *Significantly different from the control ($p< 0.05$) (Tukey test).

Table 5. Mean percentage of nuclear condensation, apoptotic bodies and nuclear communication in meristematic cells of lettuce after 72 h of exposure to Yarrow aqueous extracts.

Concentration	Nuclear alterations	
	Ncd (%)	Nb (%)
Control	1.23±0.22	0.21±0.11
Yar_1	2.65±0.65	0.27±0.14
Yar_2	18.43±3.34*	0.46±0.12*
Yar_3	33.41±4.13*	0.48±0.08*
Yar_4	-	-

Yar_1: Yarrow aqueous extract (5 mg/mL); Yar_2: Yarrow aqueous extract (10 mg/mL); Yar_3: Yarrow aqueous extract (20 mg/mL); Yar_4: Yarrow aqueous extract (30 mg/mL). Ncd: nuclear condensation; Nb: nuclear bridge. *Significantly different from the control ($p< 0.05$) (Tukey test).

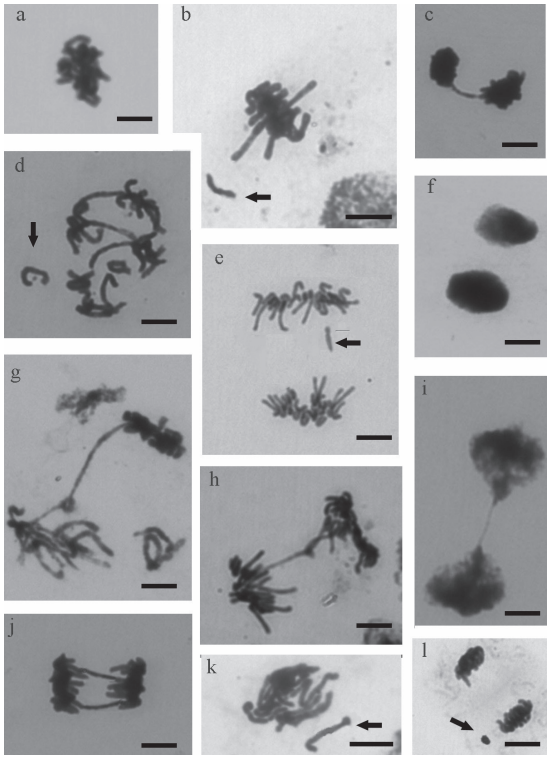

Figure 1. Chromosome aberrations observed in meristematic cells of lettuce exposed to aqueous extracts of yarrow. Stickiness metaphase (a); Metaphase with chromosome loss (arrow) (b); Telophase with bridges (c); Anaphase with bridge, polar deviation and chromosome loss (arrow) (d); Anaphase with laggard chromosome (arrow) (e), Nuclear condensation due cell death induction (f); Anaphases with bridges and chromosomes loss (g-h); Nucleus with bridge (i); Anaphases with two bridges (j); Prophase with chromosome loss (arrow) (k); Micronuclei formation (arrow) (l). Barr = 5µm.

Discussion

The mitotic index (MI), characterized by the total number of dividing cells in cell cycle, has been used as a parameter to assess the cytotoxicity of several agents. The cytotoxicity levels of an agent can be determined by the increase or decrease in the MI (Lubini et al., 2008). According to Leme & Marin-Morales (2009), MI significantly lower than the negative control can indicate alterations, deriving from the chemical action in the growth and development of exposed organisms. On the other hand, MI higher than the negative control are results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues (Campos et al., 2008). In our study, the cytological investigations indicated that root cells of lettuce in comparison to the control showed a significant decreasing of MI when Yar_2 (10 mg/mL) and Yar_3 (20 mg/mL) extracts were administered. More yet, our studies also indicated this decreasing as the principal responsible for the lower root size and little percentage of seed germinated of lettuce in these treatment. The biocide activity in the more concentrated dose (Yar_4 = 30 mg/mL) and the little development and germination of lettuce in higher concentrations of yarrow extracts are very interesting for a future utilization in the biocontrol of weed and pest development, being possible its utilization as bioherbicide. Innumerous medicinal species have demonstrated this potentiality (Negrelle & Gomes, 2007; Lubini et al., 2008; Sousa et al., 2009; 2010).

The reduction of the mitotic index in our study can be explained by the arrest of the division of the interphasic nucleus, as well as by death of interphasic nucleus, hindering the onset of the prophase and, thus, the division of the cells. In agreement of the second hypothesis, we observed various cells with cytoplasm shrinkage, nuclear condensation and apoptotic bodies and nuclear bridge, which are morphological aspects very common in the programmed cell death in plants (Solomon et al., 1999). These aspects were observed mainly in the Yar_2 (10 mg/mL) and Yar_3 (20 mg/mL) treatments, where the mitotic index showed a significant decrease in relation to the control. Similar results were observed in previous studies and cell death was the major depressor of the mitotic index (Çelik & Aslantürk, 2006; 2007; Campos et al., 2008; Lubini et al., 2008; Sousa et al., 2009; 2010).

Chromosome aberrations (CA), on the other hand, are characterized by changes in either chromosomal structure or in the total number of chromosomes, which can occur both spontaneously and as a result from exposure to physical or chemical agents (Leme & Marin-Morales, 2009). Structural chromosomal alterations may be induced by several factors, such as DNA breaks, inhibition of DNA synthesis and replication of altered DNA. The numeric CA, *e.g.* aneuploidy and polyploidy, are consequence of abnormal segregation of chromosomes, which can occur either spontaneously or by the action of aneugenic agents (Lubini et al., 2008). As in our work we observed more prominent results with CA on the highest concentration it is possible that the types of alterations are dose specific. For example, only Yar_2 and Yar_3 induced the formation of apoptotic bodies, chromosomes breakage, abnormal spindle behavior, micronuclei and nuclear communication. Thus, our results suggested caution with the use of yarrow's extracts, since some chromosome aberrations can be produced when elevated doses are administered.

In addition to the alterations discussed above, we also observed great percentage of stickiness, mainly on metaphases observed in the highest concentration (Yar_2 and Yar_3). This alteration in chromosome morphology reinforce the toxicity potentiality of some doses of yarrow extracts, once these alteration evidences the toxic effect on the chromatin, allowed by cell death (Campos et al., 2008; Lubini et al., 2008).

Micronuclei (MN) formation has been considered by many authors as the most effective and simplest endpoint to analyze the mutagenic effect promoted by chemicals (Juchimiuk et al., 2007; Pugliesi et al., 2007; Campos et al., 2008; Leme & Marin-Morales, 2009). This is due to the fact that MN results from damages, not or wrongly repaired, in the parental cells, being easily observed in daughter cells as a similar structure to the main nucleus, but in a reduced size. Thus, MN arises from the development of some CA, for instance, chromosome breaks and losses. Moreover, MN may still derive from other process as polyploidization, in which they originate from the elimination of exceeding DNA of the main nucleus in an attempt to restore the normal conditions of ploidy (Leme & Marin-Morales, 2009). In our work we didn't observed cells polyploids, nevertheless, innumerous laggard migration of the chromosomes and breaks were observed, being these alterations the principal indicators of micronuclei formation by extracts of yarrow in our study.

Based on the information provided in this work, it is concluded that the L. sativa test is a fast and sensitive assay to detect genotoxics and mutagens present in medicinal plant extracts. Moreover, for the possibility of assessing several genetic endpoints, this test also enable the evaluation of action mechanisms of the tested agents on the exposed organism's DNA. Thus, this test provides an important method for screening of mutagens and their results can be used as a warning to other test systems. More yet, the results also showed that although *A. millefolium* has a beneficial effect as a medicinal plant, serious problems and damages on cells by incorrectly usage can be observed.

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References

Alonso JR 1998. *Tratado de Fitomedicina: bases clýnicas y farmacológicas*. Buenos Aires, Argentina: Isis.

Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, Rister RS 2000.*Yarrow. Herbal Medicine Expanded Commission and Monographs*. Boston, United States: Integrative Medicine Communications.

Boswell-Ruys CL, Ritchie HE, Brown-Woodman PD 2003. Preliminary screening study of reproductive outcomes after exposure to yarrow in the pregnant rat. Birth Defects Research. *Part B. Dev Reprod Toxicol 68*: 416-420.

Bradley PR 1992. *British Herbal Compendium*. Bournemouth, United Kingdom: British Herbal Medicine Association.

Campos JMS, Davide LC, Soares GLG, Viccini LF 2008. Mutagenic effects due allelopathic action of fern (Gleicheniaceae) extracts. *Allelopath J 22*: 143-152.

Carvalho CR, Saraiva LS 1993. A new heterochromatin banding patterns revealed by modified HKG banding technique in maize chromosomes. *Heredity 70*: 515-519.

Çelik TA, Aslanturk OS 2006. Anti-mitotic and anti-genotoxic effects of *Plantago lanceolata* aqueous extracts on *Allium cepa* root tip meristem cells. *Biologia 61*: 693-697.

Çelik TA, Aslanturk OS 2007. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia 62*: 292-296.

Chacon DR, Dalla-Libera AN, Cintra DEC, Carvalho JCT, Oliveira GA, Maistro EL 2002. Absence of genotoxic and antigenotoxic effects of a standardized extract of the medicinal plant *Solanum melongena* on peripheral blood and bone marrow cells of Wistar rats. *Cytologia 67*: 417-422.

Chandler RF, Hooper SN, Hooper DL, Jamieson WD, Flinn CG, Safe LM 1982. Herbal remedies of the maritime Indians: sterols and triterpenes of *Achillea millefolium* L. (yarrow). *J Pharmacol Sci 71*: 690-693.

Correia PM 1974. *Dicionário de plantas úteis do Brasil e das exóticas cultivadas*. Rio de Janeiro, Brasil: Ministério da Agricultura e Instituto Brasileiro de Desenvolvimento Florestal.

Duke JA 1987. *Achillea millefolium* L. (Asteraceae): yarrow. In: *Handbook of Medicinal Herbs*. Florida: CRC Press, p. 9-10.

Ferreira L, Carvalho JCT, Maistro EL 2003. Standardized *Solanum melongena* extract presents protective effects against chromosomal aberrations induced by Doxorubicin in Wistar rat bone marrow cells. *Cytologia 68*: 177-181.

Guédon B, Abbe P, Lamaison JL 1993. Leaf and flower head flavonoids of *Achillea millefolium* L. subspecies. *Biochem Syst Ecol l21*: 607-611.

Haggag MY, Shalaby AS, Verzar-Petri G 1975. Thin layer and gaschromatographic studies on the essential oil from *Achillea millefolium*. *Planta Med 27*: 361-366.

Hausen BM, Breuer J, Weglewski J, Rucker G 1991. Alphaperoxyachifolid and other new sensitizing sesquiterpene lactones from yarrow (*Achillea millefolium* L. Compositae). *Contact Dermatitis 24*: 274-280.

Juchimiuk J, Hering B, Maluszynska J 2007. Multicolour FISH in an analysis of chromosomes aberrations induced by N-nitroso-N-methylurea and maleic hydrazide in barley cells. *J Appl Genet 4*8: 99-106.

Kokkalou E, Kokkini S, Handilou E 1992. Volatile constituents of *Achillea millefolium* in relation to their intraspecific variation. *Biochem Syst Ecol 20*: 665-670.

Krishna G, Kropko ML, Ciaravino V, Theiss JC 1991. *Simultaneous micronucleus* and chromosomes aberration assessment in the rat. *Mutat Res 264*: 29-35.

Kubelka W, Kastner U, Glasl S, Saukel J, Jurenitsch J 1999. Chemotaxonomic relevance of sesquiterpenes within the *Achilleamillefolium* group. *Biochem Syst Ecol 27*: 437-444.

Kuras M, Nowakowska J, Sliwinska E, Pilarsk R, Ilasz R, Tykarska T, Zobel A, Gulewicz K 2006. Changes in chromosome structure, mitotic activity and nuclear DNA content from cells of Allium Test induced by bark water extract of *Uncaria tomentosa* (Willd.) DC. *J Ethnopharmacol 107*: 211-221.

Leme DM, Marin-Morales MA 2009. *Allium cepa* test and environmental monitoring: A review on its application. *Mutat Res 682*: 71-81.

Lorenzi H, Matos FJA 2002. *Plantas Medicinais do Brasil: Nativas e Exóticas Cultivadas*. São paulo, Brasil: Instituto Plantarum, Nova Odessa.

Lubini G, Fachinetto JM, Laughinghouse HD, Paranhos JT, Silva ACF, Tedesco SB 2008. Extracts affecting mitotic division in root-tip meristematic cells. *Biologia 63*: 647-651.

Miller FM, Chow LM 1954. Alkaloids of *Achillea millefolium* L.: isolation and characterization of achilleine. *J Am Chem Soc 76*: 1353-1354.

Mitich LW 1990. Intriguing World of Weeds: Yarrow - the herb of Achilles. *Weed Technol 4*: 451-453.

Montanari T, de Carvalho JE, Dolder H 1998. Antispermatogenic effect of *Achillea millefolium* L. in mice. *Contraception 58*: 309-313.

Negrelle RRB, Gomes EC 2007. *Cymbopogon citratus* (D.C) Stapf: chemical composition and biological activities. *Rev Bras Pl Med 9*: 80-92.

Pugliesi GC, Andrade SF, Bastos JK, Maistro EL 2007. In vivo clastogenicity assessment of the *Austroplenckia populne*a (Celastraceae) leaves extract using micronucleus and chromosomes aberration assay. *Cytologia 72*: 1-6.

Sansebastian JR, Lucenti SM, Mecca D, Giglio T, Madison N, Gongliewski N, Messina D, Ciotalo VB, Matthews RJ 1990. Micronucleus test (MNT) and chromosomes aberrations (CA) from a single animal. *Environ Mol Mutagen 15*: 52.

Singh RB, Das UC 2002. Chromotoxic and mito-inhibitory effects of *Lathyrus sativus* seed extract on root tip cells of *Vicia faba. J Ecotoxicol Environ Monit 12*: 95-99.

Sobita K, Bhagirath T 2005. Effects of some medicinal plants extracts on *Vicia faba* root tip chromosomes. *Caryologia 5*8: 255-261.

Solomon M, Belenghi B, Delledonne M, Menachem E, Levine A 1999. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. *Plant Cell 11*: 431-443.

Sousa SM, Silva PS, Campos JMS, Viccini LF 2009. Cytotoxic and genotoxic effects of two medicinal species of Verbenaceae. *Caryologia 62*: 326-333.

Sousa SM, Silva PS, Viccini LF 2010. Cytogenotoxicity of *Cymbopogon citratu*s (DC) Stapf (lemon grass) aqueous extracts in vegetal test systems. *An Acad Bras Cienc 82*: 305-311.

Valant-Vetschera KM,Wollenweber E 1988. Leaf flavonoids of the *Achillea millefolium* group. Part II. Distribution patterns of free aglycones in leaf exudates. *Biochem Syst Ecol 16*: 605-614.

Zanoni FD, Andrade SF, Bastos JK, Maistro EL 2005. Clastogenicity of the *Austroplenckia populne*a (Celastraceae) bark wood extract in Wistar rat bone marrow cells. *Cytologia 70*: 303-308.

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