Cumhuriyet Üniversitesi Fen Fakültesi
Fen Bilimleri Dergisi (CFD), Cilt:36, No: 3 Özel Sayı (2015)
ISSN: 1300-1949

Cumhuriyet University Faculty of Science
Science Journal (CSJ), Vol. 36, No: 3 Special Issue (2015)
ISSN: 1300-1949

Mutagenic and Anti-mutagenic properties of the essential oil of *Jurinea leptoloba* DC by Ames Test

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Received: 01.02.2015; Accepted: 05.05.2015

Abstract. The aim of this study was to determine the mutagenic and anti-mutagenic properties of the essential oil of *Jurinea leptoloba* DC *in vitro*. The mutagenic and anti-mutagenic activities of the oil was evaluated using the *Salmonella typhimurium* tester strains TA98 and TA100, together with nitrofluorene for TA98 and sodium azide for TA100 without (-S9) metabolic activation, and 2-aminoantracene for TA98 and TA100 with metabolic (+S9) activation. The results obtained with *S. typhimurium* suggest that the oil of *J. leptoloba* can not be exploited as an anti-mutagenic agent with low adverse side effects.

Keywords: *Jurinea leptoloba*, Essential oil, Mutagenic, Anti-mutagenic

1. INTRODUCTION

The large genus *Jurinea* (*Compositae*, tribe *Cynareae*, subtribe *Carduinea*) with about 250 species is distributed in Asia and Europe [1]. Several species of *Jurinea* have been investigated chemically and presence of sesquiterpenes, especially elemanolides and melampolides have been reported [2]. The extract of the aerial parts of *Jurinea leptoloba* DC., afforded in addition to several germacranolides albicolide, pectorolide, salonitenolide, jurinelloide and its derivative, four melampolides, two elemanolides, glucopyranoside and dihydrosyringenin [3]. Previously, water-distilled essential oil from the aerial parts of *J. leptoloba* was analyzed by GC and GC-MS. Thirteen components of the oil of *J. leptoloba* characterized, representing 70.55 % of the total components detected. Non-terpenoid hydrocarbons, phenolic compounds and normal alkanes were the major components in this oil. 1,3-Menth-2-ene was identified as a main terpenoid component in this oil [4]. To the best of our knowledge no data are available about the biological and pharmacological effects of the essential oil of *J. leptoloba* in the literature. Therefore, the aims of this study were to determine the mutagenic and anti-mutagenic effects of the volatile oil of *J. leptoloba in vitro*.

2. MATERIALS AND METHODS

2.1. General

The major equipment type used was a Shimadzu UV-2501PC spectrophotometer. Microbial and cell culture media and laboratory reagents were from Merck, Germany. The mutagens 2-nitrofluorene, sodium azide and 2-aminoanhracene were also from Merck (Germany). All chemicals were of analytical grade.

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Special Issue: The Second National Conference on Applied Research in Science and Technology

2.2. Plant material

The aerial parts of *J. leptoloba* were collected in August 2011 from south of Shiraz, Iran. Voucher number 324R, deposited at the herbarium of the Department of Botany, Shahid Beheshti University, Tehran, Iran.

2.3. Isolation of the essential oil

The aerial parts of *J. leptoloba* were dried at room temperature for several days. Air-dried aerial parts of *J. leptoloba* (100g) were separately subjected to hydrodistillation using a clevenger-type apparatus for 3 h. After decanting and drying the oil over anhydrous sodium sulfate, the oil was recovered. Results showed that essential oil yield was 0.1% (w/w).

2.4. Preparation of metabolic activation system (S9 Mixture)

The S9 metabolic activator was prepared just before use by adding: phosphate buffer (0.2 M) 500µl, deionised water 130µl, KCl (0.33 M) 100µl, $MgCl_2$ (0.1 M) 80µl, S9 fraction 100µl, glucose-6-phosphate (0.1 M), 50µl and NADP (0.1 M) 40µl. The mixture was kept on ice during testing. S9 fraction, the liver post-mitochondrial supernatant of rats treated with the mixture phenobarbital/β-naphthoflavone (PB/NF) to induce the hepatic microsomal enzymes, was purchased from Moltox [5].

2.5. Toxicity determination

Bacterial toxicity was determined based on the values in Table 1. For the toxicity test, 12 ml of Nutrient agar and 0.50 ml of metabolic activation (S9) mix or Buffer (Phosphate buffer 0.2M, pH 7.4), 0.01 ml of the test chemical dilution and 0.1 ml overnight culture of the *Salmonella* strain were then added in tubes. The contents of the test tubes are then mixed and poured onto the surface of Glucose minimal agar plates. (The Glucose minimal agar, consisting of 1.5% agar, 0.02% $MgSO_4.7H_2O$, 0.2% Citric acid, 1% K_2HPO_4, 0.35% $NaNH_4HPO_4.4H_2O$ and 2% Glucose). The plates were inverted and placed in a 37°C incubator for 48 h. The colonies were then counted and the results were expressed as the number of revertant colonies per plate. Comparisons of bacterial counts on test compound plates with bacterial counts on control plates were used to determine growth inhibition [5].

Table 1. Toxicity determination.

TA100/TA98	Test 1	Control 1	Test2	Control 2
Test strain (approximately $1/6\times10^6$ cells/ml)	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Sample	0.01 ml		0.01 ml	
Phosphate buffer (0.2M, pH7.4)		0.1 ml	0.5 ml	0.6 ml
S9 mix	0.5 ml	0.5 ml		
Nutrient agar	12 ml	12 ml	12 ml	12 ml

2.6. Mutagenicity and anti-mutagenicity test

The Ames *Salmonella* mutagenicity test is a short-term bacterial reverse mutation assay specifically designed to find a wide range of chemicals that can produce genetic damage that leads to gene mutations. These mutations are the consequences of DNA damage induced by different mechanisms. When the *Salmonella* tester strains are grown on a minimal media agar

plate containing a trace of histidine, only those bacteria that revert to histidine independence are able to form colonies [5].

Mutagenic activity was evaluated by the *Salmonella*/microsome assay, using the *Salmonella typhimurium* tester strains TA98 and TA100, with (+S9) and without (-S9) metabolization, using the pre-incubation method [6]. It is important that the same number of bacteria is used in the preliminary toxicity assay as well as in the definitive mutagenicity assay [5].

Salmonella inoculated cultures 15-18 h prior to performing the experiment. Top agar melt supplemented with 0.05 mM histidine and biotin and maintain at 43°C to 48°C. To the 13×100 mm sterile glass tubes maintained at 43°C, add in the following order with mixing after each addition. Each test should be performed using a single batch of reagents, media and agar [5]. The top agar, consisting of 0.6% agar and 0.6% NaCl, is one of the most critical medium components in the Ames test because it contains the trace amount of histidine (0.05 mM) for limited growth. It also contains biotin at a concentration of 0.05 mM which is in excess of what is needed for the growth of the *Salmonella* strains. Using pre-incubation, we studied the effect of metabolic activation. In condition without metabolic activation, 0.01 ml of each concentration of test ingredient, negative control or positive control was added to 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of each strain (approximately $1/6\times10^6$ cells/ml), and then incubated at 37°C for 20 min. After shaking incubation, 2 ml of top agar was added to the incubation mixture according to the strains and then poured on to a plate containing minimal agar (Figure 1). The plates were incubated at 37°C for 48h, and the revertant colonies were counted manually. In the presence of metabolic activation, 0.5 ml of freshly prepared S9 mix instead of 0.1 M phosphate buffer (pH 7.4) were added to the incubation mixture. Other procedures were performed in the same way [5]. All experiments were performed in triplicate (Tables 2-5). The colonies are then counted, and the results are expressed as the number of revertant colonies per plate. The standard mutagens used as positive controls in experiments without the S9 mix were 2-nitrofluorene for TA98, sodium azide for TA100. In experiments with S9 activation, 2-aminoanthracene was used with TA98 and TA100. DMSO served as negative (solvent) control [5].

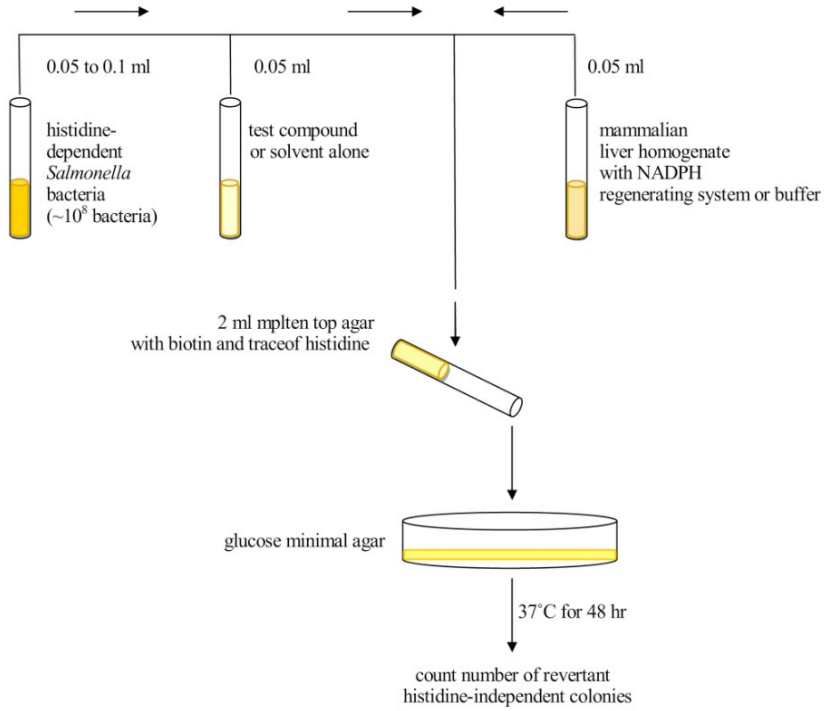

Figure 1. Diagram depicting the steps involved in the plate incorporation assay [5].

Results obtained in mutagenicity tests are shown in Tables 2,3.
The percentage of mutations was calculated using the following formula:
(T/M)*100
T: The number of revertant colonies in the presence of oil
M: The number of revertant colonies in the presence of mutagen
The number of colonies that had been grown up was deducted from the numerator and denominator.
Results obtained in anti-mutagenicity tests are shown in Tables 4,5.

Table 2. Determination of mutagenic potency of the essential oil of *J. leptoloba* by *S. typhimurium* strains TA100 and TA98 without S9.

TA100/TA98	Positive Control	Negative Control	Test
Test strain (approximately $1/6\times10^6$ cells/ml)	0.1 ml	0.1 ml	0.1 ml
sodium azide (NaN_3) (50 µg/ml) for TA100 2-nitrofluorene (1.5 µg/plate) for TA98	0.1 ml		
Test concentration			0.01 ml
Phosphate buffer (0.1 M, pH7.4)	0.5 ml	0.5 ml	0.5 ml
Top agar	2 ml	2 ml	2 ml

Table 3. Determination of mutagenic potency of the essential oil of *J. leptoloba* by *S. typhimurium* strains TA100 and TA98 with S9.

TA100/TA98	Positive Control	Negative Control	Test
Test strain (approximately $1/6\times10^6$ cells/ml)	0.1 ml	0.1 ml	0.1 ml
2-aminoanthracene (1µg/plate in DMSO)	0.1 ml		
Test concentration			0.01 ml
S9 mix	0.5 ml	0.5 ml	0.5 ml
Top agar	2 ml	2 ml	2 ml

Table 4. Determination of anti-mutagenic potency of the essential oil of *J. leptoloba* by *S. typhimurium* strains TA100 and TA98 without S9.

TA100/TA98	Positive Control	Negative Control	Test
Test strain (approximately $1/6\times10^6$ cells/ml)	0.1 ml	0.1 ml	0.1 ml
sodium azide (NaN_3) (50 µg/µl) for TA100 2-nitrofluorene (1 µg/µl) for TA98	200 µl		200 µl
Test concentration			10 µl
Phosphate buffer (0.1 M, pH7.4)	0.5 ml	0.5 ml	0.5ml
Top agar	2 ml	2 ml	2 ml

Table 5. Determination of anti-mutagenic potency of the essential oil of *J. leptoloba* by *S. typhimurium* strains TA100 and TA98 with S9.

TA100/TA98	Positive Control	Negative Control	Test
Test strain (approximately $1/6\times10^6$ cells/ml)	0.1 ml	0.1 ml	0.1 ml
2-aminoanthracene (1µg/µl in DMSO)	200 µl		200 µl
Test concentration			10 µl
S9 mix	0.5 ml	0.5 ml	0.5 ml
Top agar	2ml	2 ml	2 ml

Table 6. Percent of mutagenicity (M) and anti-mutagenicity (A) of the essential oil of *J. leptoloba* to *S. typhimurium* (TA98, TA100) with and without metabolic activation S9.

Essential oil of *Jurinea leptoloba* DC.	Dilution (mg/plate)	Percent of mutagenicity (M)			
		TA 100 M-S9	**TA 100 M+S9**	**TA 98 M-S9**	**TA 98 M+S9**
		46.94	30.00	53.26	38.57
	1.8	**Percent of anti-mutagenicity (A)**			
		TA 100 A-S9	**TA 100 A+S9**	**TA 98 A-S9**	**TA 98 A+S9**
		46.15	33.33	52.27	3.45

3. RESULTS

The concentration of the essential oil (1.8 mg/plate) was selected based on a preliminary toxicity test. The colonies were counted to determine the mutagenic and anti-mutagenic activities of *J. leptoloba* oil (Figure 2). Mutagenesis and anti-mutagenesis effects of *J. leptoloba* oil against *Salmonella typhimurium* strains TA98 and TA100, with (+S9) and without (-S9) metabolization was determined and listed in Table 6. The oil of *J. leptoloba* showed anti-mutagenic effects at the concentration of 1.8 mg/plate by the bacterial reverse mutation assay against chemical mutagens in *S. typhimurium* strains. On the other hand, mutagenesis experiments showed that the oil of *J. leptoloba* has moderate mutagenic activity in normal culture medium.

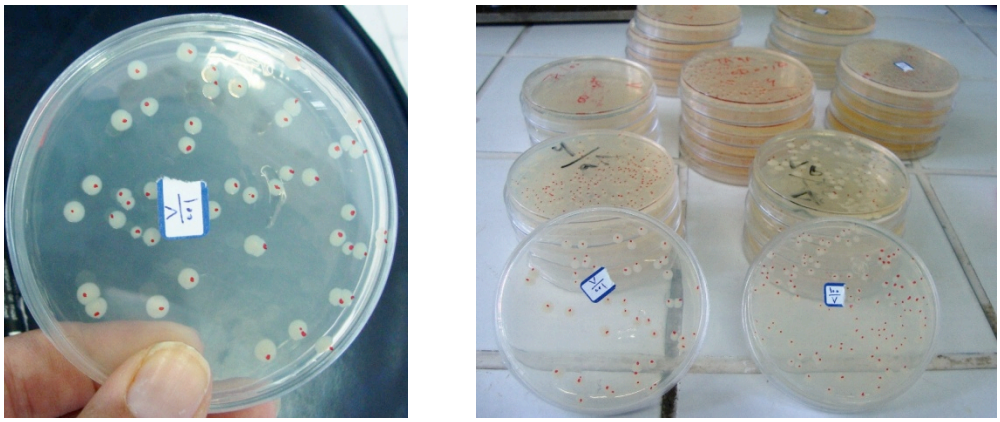

Figure 2. Colonies counting in the Ames *Salmonella* mutagenicity test.

4. DISCUSSION

There is a relationship between carcinogenesis and mutagenesis. The Ames *Salmonella* test is used to detect mutagenic and anti-mutagens activities and is a widely accepted way for identifying different chemicals and drugs that can cause gene mutations. According to the obtained results, in the presence of chemical mutagens, the oil of *J. leptoloba* showed resistance against these mutagens in both strains of *S. typhimurium* TA100 and TA98, with (+S9) and without (-S9) metabolization. On the other hand, in the absence of chemical mutagens, the oil of *J. leptoloba* showed moderate mutagenic effects in both strains of *S. typhimurium*. From the mentioned results, it can be concluded that the oil of *J. leptoloba* can not be exploited as an anti-mutagenic agent with low adverse side effects. To our knowledge, there was no research carried out on the mutagenic and anti-mutagenic effects of *J. leptoloba* oil before.

ACKNOWLEDGMENTS

The authors would like to thank sincerely Dr. Iraj Rasooli in the medicinal plant research center of Shahed University for scientific assistance. We would also like to thank Dr. Mozaffarian for help in identifying plant material. The authors wish to thank sincerely professor Rustaiyan for assistance in the botanical information.

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