Laboratoire de Biochimie et de Biologie Moléculaire[1], Laboratoire de Pharmacognosie[2], Faculté de Pharmacie, Laboratoire de Biotechnologie et Toxicologie moléculaire[3], Institut Supérieur de Biotechnologie de Monastir, Tunisie

Antimutagenic activity of aqueous extracts and essential oil isolated from *Myrtus communis*

N. Hayder[1], S. Kilani[1], A. Abdelwahed[2], A. Mahmoud[2], K. Meftahi[2], J. Ben Chibani[1], K. Ghedira[2], L. Chekir-Ghedira[1, 3]

Received October 31, 2002, accepted January 18, 2003

Pr. Leila Chekir-Ghedira, Laboratoire de Biotechnologie et Toxicologie Moléculaire, Institut Supérieur de Biotechnologie de Monastir, Av. de Environment, TUN-5019 Monastir, Tunisie
Leila.Chekir@fsm.rnu.tn

Pharmazie 58: 523–524 (2003)

Myrtus communis (Myrtaceae) is a perennial shrub, widely distributed in the Mediterranean area. In folk medicine, the leaves are used as an antiseptic and anti-inflammatory agent as well as a mouthwash for the treatment of candidiasis. The essential oil obtained from the leaves is used mainly in the treatment of lung disorders [1]. Many researchers have screened a number of antimutagenic crude principles from Chinese crude drugs [2–5]. In this study, we have found antimutagenic activity in extracts isolated from leaves of *Myrtus communis* collected from mountainous regions in Tunisia.

As shown in the Table, the three compounds tested exhibited antimutagenic activities against aflatoxin B1 (AFB1: 0.3 μg/plate) in the *Salmonella typhimurium* TA100 and TA98 assay. When 50 μg/plate of aqueous extract was added to the assay system, the number of revertants per plate for *S. typhimurium* TA100 decreased to 47% compared to those obtained in the absence of the extract. The number of TA98 revertants also showed a significant decrease of 61.6%. The inhibitory effect was further increased when the concentration of the aqueous extract was raised to 300–600 μg/plate, with inhibition rates of 91–99% respectively for *S. typhimurium* TA100. We obtained less marked inhibition rates of 61–83% with the same concentrations of aqueous extract in the *S. typhimurium* TA98 assay system. An total flavonoid oligomer (OFT) extract showed similar antimutagenic activity; the number of revertants per plate for *S. typhimurium* TA100 decreased by about 23%, 74% and 92% when 50, 300 and 600 μg/plate of OFT respectively was added to the assay system. The numbers of TA98 revertants decreased by 22%, 58% and 77% when 50, 300 and 600 μg/plate of OFT respectively was added to the assay system. However, the essential oil (EO) obtained from *Myrtus communis*, showed a less marked inhibitory effect on the mutagenicity induced by AFB1 with the TA100 assay system. 800 μg/plate of EO was necessary to obtain a mutagenic inhibiton rate of 30.4%. The highest inhibition rate obtained with EO was 58%, when 1600 μg/plate was added to the assay system. A higher dose of EO (3200 μg/plate) weakly inhibited (5.2%) the mutagenicity of AFB1 in the same assay. The antimutagenic activity of EO was higher when the TA98 assay system was used. 89% and 92% mutagenic inhibition rates were obtained when the concentration of EO was raised to 800 and 1600 μg/plate respectively, whereas 3200 μg/plate suppressed the antimutagenic effect of EO totally.

Table: Effect of aqueous extract, oligomer flavonoid enriched extract (OFT) and essential oil (EO), on the mutagenicity induced by aflatoxin B1 (AFB1, 0.3 μg/plate) in *Salmonella typhimurium* TA100 or TA98 in the presence of S9

Treatment	Concentration (μg/plate)	Revertants per plate ± SD	
		TA100	TA98
Spontaneous	–	152 ± 2	60 ± 7
AFB1	–	530 ± 10	730 ± 5
Aqueous extract	50	352 ± 2	317 ± 7
	300	186 ± 4	316 ± 8
	600	155 ± 5	170 ± 3
OFT	50	442 ± 12	580 ± 3
	300	205 ± 5	340 ± 8
	600	181 ± 1	210 ± 3
EO	800	415 ± 15	132 ± 3
	1600	310 ± 10	112 ± 8
	3200	510 ± 10	740 ± 5

In the present study, the antimutagenic effects of the aqueous extract, the OFT extract and essential oil of *Myrtus communis* leaves were observed in both TA100 and TA98, which are *S. typhimurium* strains showing respectively a base-pair substitution mutation in gene G of the histidine operon, and a frame-shift mutation due to the lack of a base pair in the GC-pair region of gene D [6]. The above observations suggest that the tested extracts exert an influence on hereditary factors. The antimutagenic activity of the OFT extract was lower (92.3%) compared to the mutagenicity inhibition rate obtained with the aqueous extract (99.2%). This result correlates with the chemical composition of the two extracts which revealed the presence of important quantities of tannins and flavonoids in the aqueous extract [6–8], whereas the OFT extract contains a lower quantity of tannins and about the same quantity of flavonoids, compared to the aqueous extract. The results of our experiments confirm the known antimutagenic activity of flavonoids [4, 9] and tannins [5, 10–12] extracted from different plants. Antimutagenically active substances have been found mainly in various phenolics e.g. flavonoïds, coumarins, tannins, xanthones and quinones [2]. On the other hand essential oil showed an antimutagenic effect against the indirectly acting mutagen AFB1, although a large excess quantity was necessary. The antimutagenic effect of EO nearly disappeared when a large excess of this extract was added to the assay system (5.2% with 3200 μg/plate). We postulate that an excess of EO could inhibit the penetration into the cell of molecules which are implicated in the mutagenic inhibitory effect towards AFB1. EO contains neither tannins nor flavonoids, so this could explain its low antimutagenic effect.

In conclusion, we assume that the inhibitory effects of the species reported in this paper are mainly due to both the tannin and flavonoid fractions. This is supported by the weak inhibitory effect obtained with the essential oil which is an extract free of tannins and flavonoids. We cannot however exclude the possibility that other phenolic or non phenolic compounds with antimutagenic properties are concerned with the mutagenic inhibitory effect of the extracts obtained from leaves of *Myrtus communis*.

Experimental

Myrtus communis var *italica* was collected from the Boukornine National Parc situated in the North East of Tunisia in November 1998.

The powdered leaves were extracted with boiling water for 15 to 20 min. After filtration, the extracts were filtred and lyophilized (aqueous extract). In order to obtain extracts enriched with total flavonoids oligomers (OFT), we macerated the powdered leaves in a water/acetone mixture (1v/2v), for 24 h with continuous stirring. The extract was filtered and the acetone was evaporated under low pressure in order to obtain an aqueous phase. The tannins were removed by precipitation with an excess of NaCl for 24 h at 5 °C, and we then recovered the supernatant. This latter was extracted with ethylacetate, concentrated and precipitated with an excess of chloroform. The precipitate was separated and yielded the OFT extract. Essential oils were extracted by steam distillation [13] of fresh leaves of *Myrtus communis*.

Mutagenesis assays were performed with *Salmonella typhimurium* TA100 and TA98, kindly provided by Prof. Quillardet, (Institut Pasteur, Paris, France). The assay was performed with an exogenous metabolic system; the S9 fraction in S9 mix. The S9 fraction was prepared according to the method described by Maron and Ames [14] with some modifications [15]. The metabolic activation system was prepared by addition of 10% of the S9 fraction just prior to use according to Maron and Ames [14] and kept in ice during the test.

A modified plate incorporation procedure [2] was employed to determine the effect of all isolates (hydrosoluble extracts or essential oils) on AFB1 induced mutagenicity. The mutagenicity inhibition rate (%) was calculated relative to the control group with the mutagen by the following equation: Inhibition rate (%) = [1-(number of revertants on test plates/number of revertants on control plates)] $\times$ 100. Toxicity tests for different levels of samples were also carried out, and the sample concentrations employed for the antimutagenic test showed no toxicity to the test strains. Data were collected with a mean $\pm$ standard deviation of three plates (n = 3).

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