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Antioxidant activity and inhibition of aflatoxin B_1-, nifuroxazide-, and sodium azide-induced mutagenicity by extracts from *Rhamnus alaternus* L.

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ABSTRACT

The effect of extracts obtained from *Rhamnus alaternus* L. leaves on genotoxicity and SOS response induced by aflatoxin B_1 (10 μg/assay) as well as nifuroxazide (20 μg/assay) was investigated in a bacterial assay system, i.e., the SOS chromotest with *Escherichia coli* PQ37. The evaluation of the mutagenic and antimutagenic actions of the same extracts against the sodium azide (1.5 μg/plate)-induced mutagenicity was assayed using the *Salmonella typhimurium* assay system. The *R. alaternus* tested extracts exhibited no genotoxicity either with or without the external S9 activation mixture. However, all the extracts, particularly aqueous extract (A) and its chloroformic fraction (A_2) significantly decreased the genotoxicity induced by aflatoxin B_1 and nifuroxazide. Moreover, the different extracts showed no mutagenicity when tested with *Salmonella typhimurium* strains TA1535 and TA1538 either with or without the S9 mix. Aqueous extract as well as its A_2 fraction exhibited the highest level of protection towards the direct mutagen, sodium azide-induced response in TA1535 strain with mutagenicity inhibition percentages of 83.6% and 91.4%, respectively, at a dose of 250 μg/plate. The results obtained by the Ames test assay confirm those of SOS chromotest. These same active extracts exhibited high xanthine oxidase (XOD) inhibiting with respective IC_{50} values of 208 and 137 μg/ml, and superoxide anion-scavenging effects (IC_{50} values of 132 and 117 μg/ml) when tested in the XOD enzymatic assay system. Our findings emphasize the potential of *R. alaternus* to prevent mutations and also its antioxidant effect.

1. Introduction

Exposure to genotoxic chemicals present in food, in the environment and used in medical treatment can alter the genetic material permanently and thus may lead to cancer [1]. At present, there are several antigenotoxicity assays available, which include the micronucleus test, somatic mutation and recombination test (SMART), sister chromatid exchange (SCE) assay and the single cell gel electrophoresis (SCGE) or comet assay. The above-mentioned assays may involve a longer analysis time, a high cost, and specialized skill or may require addition of expensive reagents. Therefore, short-term bacterial assays: Ames test and the SOS chromotest assay are useful and give an

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estimation of the genotoxic/antigenotoxic potential of substances [2].

Antigenotoxic plant extracts can counter or prevent the adverse effects caused by DNA damaging chemicals. In fact, researches, however, have shown that plant-derived polyphenolic compounds exert antigenotoxic property [3,4].

Accumulating data from *in vitro* and short-term *in vivo* studies as well as long-term carcinogenicity studies with chemically treated animals continue to show that phytochemicals could possess antimutagenic and anticarcinogenic effects [5]. Additionally, epidemiological studies support that chemopreventive effects are associated with the intake of plant materials [6]. However, under some experimental conditions, these phytochemicals exhibit genotoxic and/or mutagenic effects by themselves or potentiate the effect of other xenobiotics [7]. Thus it is of paramount importance to investigate the circumstances under which phytochemicals, used in traditional medicine exhibit beneficial and harmful effects [8].

On the other hand, oxidative stress, caused by reactive oxygen species (ROS), is known to cause the oxidation of biomolecules leading to cellular damage. The tissue injury caused by ROS may include DNA and protein damages, and oxidation of important enzymes. These events could consequently lead to the occurrence of various free radical-related diseases. In the human body, the toxic effects of ROS are combated regularly by a number of endogenous defence and protective mechanisms which include various enzymes and non-enzymatic antioxidants. These self-defence systems may also be supported by antioxidative compounds taken as foods, cosmetics and herb medicine particularly in the elderly [9].

Recently, natural foods and food derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention, because they are known to function as chemopreventive agents against oxidative damage. Fruits, vegetables and herb medicines contain many antioxidant compounds, including carotenoids, thiols, vitamins such as ascorbic acid, tocopherols, flavonoids, and other phenolics [10].

The plant *R. alaternus* L. (Rhamnaceae), a wild species of the Mediterranean region, is commonly found growing in wilds around the farmlands in the North of Tunisia. It is known locally as "Oud el-Khir". The sheet of *R. alaternus* is laxative, purgative and has a strongly hypotensive activity [11]. The decoction of the air parts of this plant seems to be effective in the treatment of the hepatic complications while the bark in a meat bubble is employed in the North Africa countries against certain dermatological and hepatic affections. Other reported indications are the treatment of the burns and odontology and for the ocular care [12,13]. At present, scientific information reported on chemical and biological properties of *R. alaternus* remains limited. From the literatures, this plant species is known to contain compounds such as emodin, alaternin, quercetin, kaempferol, chrysophanol and physcion [14,15]. Although this plant species has been widely used as folk medicine in Tunisia and many other North African countries, it remains unknown if certain therapeutic claims of the *R. alaternus* preparations are derived from its antioxidant and/or antigenotoxic activities.

In this study, our aim was to examine the antimutagenic and antigenotoxic capacities of different *R. alaternus* extracts using SOS chromotest and Ames test assays. The objective here was also, to evaluate the antioxidant capacity of the *R. alaternus* extracts by the XOD system generated superoxide anion radical inhibition assay.

2. Materials and methods

2.1. Plant material

The leaves of *R. alaternus* were collected in the region of Tabarka situated in the western North of Tunisia in November 2006. Botanical identification was carried out by Dr. Ben Tiba (Institut Supérieur d'Agronomie, Chott-Mariam, Tunisia), according to the flora of Tunisia [16]. A voucher specimen (Ra-12-004) has been deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy of Monastir, Tunisia. The leaves were shade dried, powdered and stored in a tightly closed container for further use.

2.2. Preparation of plant extracts

The fresh leaves of *R. alaternus* L. were dried at room temperature and reduced to coarse powder. One hundred grams of the powdered leaves were extracted with boiling water (1 L) for 15–20 min. After filtration, the crude extract obtained was frozen and lyophilized, leading to the aqueous extract (A) which was dissolved in water. A_1, A_2 and A_3 were respectively the chloroformic, ethyl acetate and butanolic fractions of the aqueous extract, obtained by a liquid–liquid separation. These fractions were concentrated to dryness and the residues were kept at 4 °C. Then, they were dissolved in dimethyl sulfoxide (DMSO).

Petroleum ether (PE) and Chloroform ($CHCl_3$) extracts were obtained by Soxhlet extraction (6 h) using 100 g of the powdered leaves and 1 L of solvent. These two extracts, with different polarities, were also concentrated to dryness and the residue was kept at 4 °C. They were dissolved in DMSO.

In the present study, six extracts were investigated. The doses of extracts we tested in both *Salmonella* microsome assay (10, 50, 250 and 350 μg/plate), SOS chromotest (10, 50 and 250 μg/plate) and xanthine/xanthine oxidase enzymatic assay (50, 150 and 300 μg/ml, respectively) are in accordance with our previous investigations [17–21], where a number of preliminary dose-finding tests involving a number of plant extracts were conducted. This means that the doses were suitable for testing the majority of the extracts, however, not necessarily all. Therefore, some extracts may be toxic at one or more of the applied doses.

2.3. Chemicals

O-Nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-nitrophenylphosphate (PNPP) were purchased from Merck (Dramstadt, Germany). The positive mutagens AFB_1, nifuroxazide and sodium azide (SA) were purchased from Sigma–Aldrich (St. Louis, MO, USA); histidine, biotine and agar–agar from Difco (Detroit, MI, USA); Aroclor 1254 from