Antiviral activity of leaves extracts of *Marrubium alysson* L.

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Petroleum ether, ethyl acetate, chloroform, butanol and methanol extracts of the leaves of *Marrubium alysson* L. were screened for *in vitro* antiviral activity against human cytomegalovirus (HCMV) strain AD-169 (ATCC Ref. VR 538) and coxsackie B virus type 3 (CoxB-3) using a cytopathic effect (CPE) reduction assay. Total phenolic content of the extracts were determined by the Folin-Ciocalteu colorimetric method. The most potent inhibition was observed with methanol extract, which inhibited HCMV and Cox B3 viruses replication at 100 µg/ml without showing cytotoxic effects. Good activities were also found with the ethyl acetate, butanol and chloroform extracts which exhibited antiviral effect against both HCMV and Cox B3 at 100 to 250 µg/ml.

Key words: *Marrubium alysson* L., antiviral activity, total phenolic content.

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. There has been growing interest in the investigation of the natural products from plants for the discovery of new antimicrobial agents. The appearance of viral resistant strains to antiviral agents is an emerging problem. Thus, the prevalence of virally related diseases is of growing concern. For that reason, the development of new and better antiviral compounds is vital and desirable. It has been demonstrated that natural products are preferable to synthetic compounds as sources of new antiviral agents (Vanden Berghe et al., 1986; Vlietinck et al., 1995; Abad et al., 1997). A number of compounds with inhibitory activity against the replication of several viruses have been extracted from a number of medicinal plants (De Rodriguez et al., 1990; Marchetti et al., 1996; Hayashi et al., 1997).

The genus Marrubium is represented by 97 species which are widely spread over the temperate and warm regions. Many Marrubium species are reported in the literature to be used in folk medicine (Anastasia et al., 2003, Anastasia et al., 2005). Most of the species are annual or rhizomatous perennial herbs with a distinct indumentums of often very complex hairs, and verticillasters subtended by floral leaves. *Marrubium alysson* is a common plant in Tunisia and is used in traditional medicine in the form of a decoction as a remedy for asthma, diabetes and as a diuretic. Recent research demonstrated the antibacterial activity of *M. alysson* extracts (Edziri et al., 2007). To the best of our knowledge the antiviral activity of *M. alysson* has not been studied yet.

The aim of this study was to investigate the antiviral
property of methanol extracts of *M. alysson* L. growing in Tunisia.

**MATERIALS AND METHODS**

**Plant materials**

Fresh aerial parts were collected in 2010 in kerker (Tunisian centre). The plant was identified by Pr. Mohamed Chaieb botanist in the University of Science (Sfax, Tunisia). A voucher specimen was deposited in the herbarium of our laboratory.

**Extraction**

750 g of dried *M. alysson* were extracted with MeOH through maceration for five days. The resulting extract was evaporated at reduced pressure to obtain a green residue (279 g) which was successively fractionated with, petroleum ether, chloroform and ethyl acetate and these fractions were used for biological tests.

**Determination of total phenolic content**

Total phenolic content in each extract was determined using Folin-Ciocalteus reagent according to the method of Singleton et al. (1965). Forty microliters of extract (1 mg/ml) was mixed with 200 µl distilled water, followed by 600 µl 20% sodium carbonate (Na₂CO₃) 3 min later. The mixture was shaken for 2 h at room temperature and absorbance was measured at 765 nm. All tests were performed in triplicate. Gallic acid (Sigma-Aldrich, Germany) was used as a standard. The concentration of total phenolic compounds (TPC) was determined as µg gallic acid equivalents (GAE)/mg extract using the following equation obtained from a standard gallic acid graph (R² = 0.9877):

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\text{Absorbance} = 0.0012 \times \text{gallic acid (µg)} - 0.0034
\]

**Antiviral activity assay**

We have adopted a cytopathic effect (CPE) reduction assay for screening the antiviral activities of the plant extracts. In brief to confluent cell monolayers in a 96-well plate, 100 TCID₅₀ (50% tissue culture-infective dose) virus suspension and serial two-fold dilutions of crude extracts were added simultaneously. As positive control, cells were infected with the same concentration of virus but without the addition of extract, and as a negative or cell control, only MEM-D was added to the cells. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 3 to 5 days. The concentration that reduced 50% of CPE in respect to the virus control was estimated from the plots of the data and was defined as the 50% inhibitory concentration (IC₅₀). The selective index (SI) was calculated from the ratio CC₅₀/IC₅₀ (Kujumgier et al., 1999).

**RESULTS AND DISCUSSION**

The TPC was expressed in equivalent of gallic acid (mg CE/g of extract). The total phenolic content of extracts of *M. alysson* L. is given in Figure 1. The total phenolic content varied from 123.98 to 26.6%. We can conclude that methanol and butanol extracts had the highest total phenolic contents than the other extracts. The antiviral activity was estimated on the basis of cytopathic effect (CPE) of the virus-infected confluent monolayer of MRC 5 cells. The mean IC₅₀, CC₅₀ and SI (CC₅₀/IC₅₀) values are shown in Table 1. We can conclude that all extracts were not toxic against MRC5 cells (IC₅₀ > 500 µg/ml). The most active extract was the methanol extract, which inhibited HCMV and Cox B3 viruses replication at 100 µg/ml without showing cytotoxic effects and with selective index upper to 5. Good activities were also found with the ethyl acetate, butanol and chloroform extracts which exhibited antiviral effect against both HCMV and Cox B3 viruses at concentrations ranging from 100 to 250 µg/ml.

Viral infections are accompanied by profound changes in cell/tissue metabolism, which lead to severe generation of reactive oxygen species. The latter may cause aggravation in the pathogenesis of the infection. It has been found recently that the main cause of mortality from
influenza virus-induced pneumonia is the cytotoxicity, which is determined by the considerably increased levels of O2 before the viral replication in the bronchial epithelial cells (Akaike et al., 1996). Therefore the use of antioxidants could be of great value in preventing the inception or the progression of the disease to play an important role in the mechanism of antiviral activity to reduce the action of reactive oxygen species (ROS) in tissue damage.

Methanol, butanol and ethyl acetate extracts showed significant anti-Coxsaki B3 activity. This observed activity may be due to the higher amount of phenolic compounds particularly flavonoids and tannins known to possess good antiviral activities (Fukuchi et al., 1989; Namba et al., 1998).

The substances responsible for the antiviral activity must be isolated and chemically characterized. Further analysis, including additional purification of the extracts, along with further antiviral testing, are currently being conducted.
REFERENCES


