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# Screening of selected Arabian medicinal plant extracts for inhibitory activity against peptidases

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The methanolic extracts of 20 medicinal plants from the island Soqotra/Yemen were screened with respect to their inhibitory potency against angiotensin converting enzyme (ACE), neutral endopeptidase (NEP) and aminopeptidase N (APN). Eight extracts did not show significant inhibitory activity against the enzymes tested, only *Kalanchoe farinacea*, *Boswellia elongata* and *Cissus hamaderohensis* inhibited all three enzymes. The most active extract was prepared from *Kalanchoe farinacea* characterized by low $IC_{50}$ values especially for NEP and APN.

## 1. Introduction

Herbal medicine represents one of the most important fields of traditional medicine in Yemen, predominately in rural areas. Thus, phytotherapy is practiced by a large proportion of Yemen population to heal several physical, physiological, mental and social ailments. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs it is essential to study medicinal plants, which have folklore reputation, in a more intensified way (Mothana and Lindequist 2005).

Neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) belong to the group of zinc-endopeptidases acting as ectoenzymes on the outer surface of different cells. Both peptidases are included in the metabolism of regulatory peptides, like substance P, bradykinin, enkephalins, natriuretic peptides and other vasoactive and hormonal peptides. Scientific research focused on the role of NEP in inactivating the natriuretic peptides *in vivo* (Skidgel and Erdös 2004). Moreover, the availability of circulating or tissue natriuretic peptides may be increased by inhibiting its metabolic clearance by NEP-inhibitors. These agents induce a reduction in blood pressure and diuretic effects in animal models, and may become a new class of drugs for the clinical management of patients with hypertension and congestive heart failure. The effects of NEP-inhibitors are synergistically supported by inhibitors of ACE. While simultaneously inhibiting the renin-angiotensin-aldosterone system and stimulating the natriuretic peptide and kinin system, inhibitors of NEP and ACE reduce vasoconstriction, enhance vasodilatation, improve sodium/water balance, and, in turn, decrease peripheral vascular resistance as well as blood pressure and improve local blood flow (Floras 2002). Aminopeptidase N (APN) is also included in the inactivation of a variety of biological active peptides acting with NEP and ACE synergistically (Albiston et al. 2004). The importance of ACE and NEP as targets in therapeutic approaches requires new inhibitors of both enzymes, so called vasopeptidase inhibitors (Meier et al. 2005).

## 2. Investigations, results and discussion

The screening of 20 methanolic extracts from medicinal plants sampled on the island Soqotra/Yemen for the inhibitory activity against three peptidases included in regulation of different physiological and pathological processes in humans showed that twelve of the test extracts were able to inhibit the enzymatic activity of the peptidases (Table). Eight extracts were only weak inhibitors of the enzymes, showing inhibition rates smaller than 50%, while concentrations of 400 μg/ml were tested, i. e. no $IC_{50}$ could be calculated. Three extracts, those from *Kalanchoe farinacea*, *Boswellia elongata* and *Cissus hamaderohensis*, inhibited the activity of all three peptidases.

The inhibition of NEP, ACE and APN has beneficial effects regarding the treatment of bronchitis and asthma (Maclean et al. 2000; O'Connor et al. 2004). The inhibitory activities of *Boswellia elongata* extracts against NEP and ACE correspond with the use of this plant in Arabic traditional medicine for diseases of the respiratory tract (Reiß 2003). For *Cissus hamadorensis* and *Kalanchoe farinacea* an use in treatment of wounds, skin diseases and inflammation in Arabic folk medicine has been reported (Miller and Morris 1988). Especially, the inhibition of APN activity might correlate with that use, because this enzyme is included in the regulation of the inflammation metabolism (Gabrilovac et al. 2005). The inhibition of NEP activity by more than 50% of the extracts tested is in accordance with reports about the use of all these plants to cure diarrhoea. Inhibitors of NEP activity cause constipation. Some of them, e.g. racecadotril, are used in modern medicine also for the treatment of bacterial induced diarrhoea (Alam et al. 2003; Wang et al. 2005). There are no reports considering the utilisation of the medicinal

**Table: Effect of different methanolic extracts of Arabian medicinal plants on peptidase activities**

| Plant | Part for extraction | $IC_{50}$ ACE (μg/ml) | $IC_{50}$ NEP (μg/ml) | $IC_{50}$ APN (μg/ml) |
|---|---|---|---|---|
| *Boswellia ameero* Balf.f. | Bark | >400 | 19 | 69 |
| *Boswellia elongata* Balf.f. | Bark | 292 | 9 | 69 |
| *Buxus hildebrandtii* Baill. | Aerial plant material | >400 | >400 | >400 |
| *Cassia socotrana* Serrato | Aerial plant material | >400 | >400 | >400 |
| *Cissus hamaderohensis* Radclife-Smith | Leaves | 391 | 26 | 57 |
| *Cissus subaphylla* (I.B.Balf.) Planch | Leaves | >400 | 303 | >400 |
| *Dendrosicyos socotrana* Balf.f. | Aerial plant material | >400 | >400 | >400 |
| *Dracaena cinnabari* Balf.f. | Leaves and flowers | >400 | 270 | >400 |
| *Euryops arabicus* Steud. ex Jaub. & Spach | Aerial plant material | >400 | 420 | >400 |
| *Exacum affine* Balf.f. | Aerial plant material | 312 | 330 | >400 |
| *Fagonia luntii* Baker | Aerial plant material | >400 | 374 | >400 |
| *Gnidia socotrana* Gilg | Aerial plant material | >400 | 69 | 327 |
| *Jatropha unicostata* Balf.f. | Leaves and fruits | 180 | 154 | >400 |
| *Jatropha unicostata* Balf.f. | Bark | >400 | 188 | >400 |
| *Kalanchoe farinacea* Balf.f. | Aerial plant material | 87 | 7 | 2 |
| *Pulicaria stefanocarpa* Balf.f. | Aerial plant material | 333 | 209 | >400 |
| *Trichocalyx obovatus* Balf.f. | Aerial plant material | 420 | >400 | >400 |
| *Withania adunensis* Balf.f. | Aerial plant material | >400 | >400 | >400 |
| *Withania riebeckii* Schweinf.ex Balf.f. | Aerial plant material | >400 | >400 | >400 |
| *Zygophyllum quatarense* M.N.Hadidi | Aerial plant material | >400 | >400 | >400 |

plants investigated to cure patients with hypertension and congestive heart failure. Presumably, these disorders are not a problem in this part of Arabia and/or no therapeutic tradition has been developed for the treatment of cardiovascular diseases. One proof for this assumption is the most interesting plant from the investigated extracts, *Kalanchoe farinacea* Balf.f., from which low $IC_{50}$ values for the enzyme activities were determined. There are scarcely reports about phytochemistry and their use in traditional medicine, only an antimicrobial activity is published (Mothana and Lindequist 2005). It is known that several *Kalanchoe* species contain bufadienolides from the bryophylline-type (Teuscher and Lindequist 1994), but there are no reports about therapeutic use for cardiac insufficiency. Because of the importance of vasopeptidase inhibitors the isolation and characterization of the natural products responsible for the inhibitory activities might be a source for new structures usable for the development of substances applicable to cardiovascular disorders or for diarrhoea.

## 3. Experimental

### *3.1. Materials*

The plants were collected from different localities on the island Soqotra (Yemen) in the beginning of spring as well as in the winter 2002 and identified at the Botany Department, Faculty of Science, Sana'a University. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

L-Leucine-p-nitroanilide, Hip-L-His-L-Leu, Suc-L-Ala-L-Ala-Phe-7-amino-3-methyl-coumarin (SAAP-AMC), phosphoramidon and aminopeptidase (leucine aminopeptidase, type IV-S from porcine kidney microsomes) were purchased from Sigma. *o*-Phthalaldehyde was obtained from Merck. Lisinopril was a gift of Schering & Plough (USA). Source of NEP and ACE was a boar sperm preparation provided by Dr. Siems (Research Institute for Molecular Pharmacology, Berlin, Germany).

### *3.2. Extraction of plant material*

The air-dried and powdered plant materials (10 g of each) were extracted successively under shaking with chloroform for three to five times at room temperature and then with 90% methanol in a water-bath at 50 °C for three to five times. The obtained extracts were filtered and evaporated in a vacuum evaporator or freeze dryer to give the dried extract. Only the methanolic extracts were used in the experiments described.

### *3.3. Determination of enzyme activity*

Enzyme activities were determined according to Bormann and Melzig (2000).

For ACE a Hip-L-His-L-Leu solution (20 μl, 4 mM in water) was added to 30 μl of phosphate-buffer (83 mM $K_2HPO_4$ + 326 mM NaCl, pH 8.3), 50 μl of extract solution and 200 μl of ACE (1 : 300). The reaction was incubated for 30 min (37°) than stopped with 0.4 M NaOH (1000 μl), and methanolic *o*-phthalaldehyde solution (2%, 100 μl) was added for producing the fluorescence His-Leu-*o*-phthalaldehyde complex. Under exclusion of light this mixture was incubated for 10 min and terminated by addition of 2 M HCl (300 μl). The fluorescence was measured at 365 $nm_{exit}$/500 $nm_{emiss}$. The inhibition rate was calculated in comparison to the control without inhibitor, considering the absorbance of fluorescence light from the test extracts. As positive control we used lisinopril (1 μM) which inhibited the enzymatic reaction completely.

For determination of the NEP activity a two-step assay was used. Lisinopril (50 μl, 8 μM), 50 μl SAAP-AMC (400 μM) and 350 μl HEPES-buffer (50 mM + 154 mM NaCl, pH 7.4) with and without extracts were added and mixed. The first enzymatic reaction was started by adding 150 μl of diluted (1 : 1000) boar sperm preparation and incubated for 60 min (37°C). The reaction was stopped by an addition of 50 μl phosphoramidon solution (50 μM). 20 μl of APN-solution (1 : 235) were supplied and the reaction mixture was incubated again for 60 min. (56°C). The reaction was terminated by an addition of 800 μl acetone. The fluorescence of the released AMC was measured at 367 $nm_{exit}$/440 $nm_{emiss}$. The inhibition rate was calculated in comparison to the control solution without inhibitor, considering the possibility of an influence on APN or/and fluorescence by test extracts. Phosphoramidon was used as the positive control (1 μM) which inhibited the enzymatic reaction completely.

For determination of the activity of APN L-leucine-*p*-nitroanilide solution (250 μl, 2 mM in HEPES-buffer) was added to 200 μl of HEPES-buffer (50 mM + 154 mM NaCl, pH 7.4) with and without test extracts. The reaction was started by adding 50 μl of APN solution (1 : 5000 in HEPES-buffer) and the solution was incubated for 60 min (37°C). Supplying 800 μl acetone stopped the reaction. The samples were measured spectrophotometrically at 405 nm to determine the *p*-nitroaniline formed.

$IC_{50}$ values were obtained from dose-effect curves by linear regression.

Test compounds were dissolved in DMSO and then diluted. The influence of DMSO (lower than 0.1%) was considered based upon control samples.

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