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Pumpkin seed extract: Cell growth inhibition of hyperplastic and cancer cells, independent of steroid hormone receptors

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ABSTRACT

Pumpkin seeds have been known in folk medicine as remedy for kidney, bladder and prostate disorders since centuries. Nevertheless, pumpkin research provides insufficient data to back up traditional beliefs of ethnomedical practice. The bioactivity of a hydro-ethanolic extract of pumpkin seeds from the Styrian pumpkin, *Cucurbita pepo* L. subsp. *pepo* var. styriaca, was investigated. As pumpkin seed extracts are standardized to cucurbitin, this compound was also tested. Transactivational activity was evaluated for human androgen receptor, estrogen receptor and progesterone receptor with *in vitro* yeast assays. Cell viability tests with prostate cancer cells, breast cancer cells, colorectal adenocarcinoma cells and a hyperplastic cell line from benign prostate hyperplasia tissue were performed. As model for non-hyperplastic cells, effects on cell viability were tested with a human dermal fibroblast cell line (HDF-5). No transactivational activity was found for human androgen receptor, estrogen receptor and progesterone receptor, for both, extract and cucurbitin. A cell growth inhibition of ~40–50% was observed for all cell lines, with the exception of HDF-5, which showed with ~20% much lower cell growth inhibition. Given the receptor status of some cell lines, a steroid-hormone receptor independent growth inhibiting effect can be assumed. The cell growth inhibition for fast growing cells together with the cell growth inhibition of prostate-, breast- and colon cancer cells corroborates the ethnomedical use of pumpkin seeds for a treatment of benign prostate hyperplasia. Moreover, due to the lack of androgenic activity, pumpkin seed applications can be regarded as safe for the prostate.

1. Introduction

Pumpkins are known all over the world and belong to the family Cucurbitaceae, which includes economically very important species. Aqueous extracts of pumpkin seeds are used in folk medicine to treat urinary tract complications. Hydroethanolic pumpkin seed extracts showed promising activities on stress urinary incontinence, on urination frequency and on nocturia in clinical trials, which draw the research interest to the polar part, instead of the more well-known seed oil [1–4].

Despite the widespread use, effects on cell growth and receptor interaction are not known. Pumpkins are consumed mostly as food, but Cucurbitaceae representatives are also used in several countries such as Mexico, North India, China, and in the Caribbean, in ethnomedicinal applications [5–9]. In Central Europe, pumpkin seeds are recommended for bladder and prostate problems and several dietary supplements are commercially available for this purpose. Pumpkins are also used for prostate problems in the Caribbean area [7]. In Austria a special variety, the Styrian oil pumpkin, *Cucurbita pepo* L. subsp. *pepo* var. styriaca, had been grown for decades [10] and is also used as food supplement.

Nevertheless, the bioactive properties of pumpkins have been barely investigated. In addition, pumpkin seeds are often tested in a complex mixture with other plants and ingredients. For example, Jiang et al. [11,12] demonstrated inhibition of prostate cancer *in vitro* and in a xenograft model with a polyherbal dietary supplement where pumpkin seeds are only one of 33 ingredients. This makes an interpretation of the efficacy of individual extracts and compounds impossible.

In a randomized, double-blind, placebo-controlled trial with 47 benign prostatic hyperplasia patients, international prostate symptom score (IPSS) was reduced by a 3 months treatment with pumpkin seed oil, but prostate specific antigen and prostate volume were not reduced [13]. IPSS is a standardized questionnaire that is used to evaluate symptoms of benign prostate hyperplasia. Another clinical trial with 2245 patients with benign prostate hyperplasia, who received during 3 months an ethanolic pumpkin seed extract, resulted also in an efficient improvement of BPH symptoms, especially in early stages, which was measured with a decreased IPSS [14].

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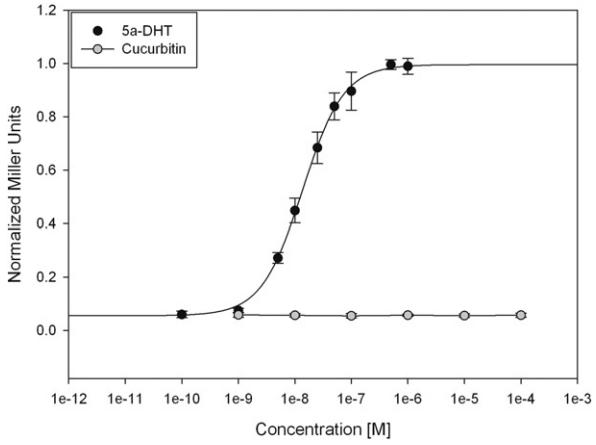

Fig. 1. Cucurbitin in yeast androgen assay. 5-alpha-Dihydrotestosterone (5a-DHT) was the reference compound and DMSO was used as negative control. Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

Beside those studies, only a few *in vivo* experiments were conducted, but mostly with pumpkin seed oil; these studies show a benevolent impact on prostate *via* inhibition of testosterone-induced hyperplasia [15, 16].

Another possibility to prevent an immense growth of hyperplastic tissue could be obtained by cytotoxic effects. Cytotoxic activity was reported for fig-leaf gourd (*Cucurbita ficifolia*), a Cucurbitaceae species that is common in Mexico [17]. This was also reported for compounds such as cucurbitacins, moschatin and cucurmosin from other Cucurbitaceae species [18–21].

Nevertheless, these studies present only a small glimpse in the potential of Cucurbitaceae as therapeutic and do not explain satisfactorily traditional ethnomedical applications. More studies are needed to corroborate ethnopharmacological records or to dismiss them. In this study, we tested two effects of pumpkin seed extract and cucurbitin, (*3R*)-3-aminopyrrolidine-3-carboxylic acid, that is used to standardize this extract. In the first place the transactivation of human androgen receptor, estrogen receptor α and progesterone receptor has been tested, and secondly the impact on cell viability of several cancer cell lines, a hyperplastic cell line and a normal fibroblastic cell line has been evaluated. Experiments with an androgen-sensitive and an androgen-insensitive prostate cancer cell line were performed to test if possible effects are mediated by an androgen-dependent route. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

2. Materials and methods

2.1. Chemicals and media

Dimethyl sulfoxide (DMSO), 17β-estradiol (E2), β-naphthoflavone, 5α-dihydrotestosterone (5α-DHT), *N*-lauroylsarcosine (sodium salt), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 * 2\ H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 * 2\ H_2O$), potassium chloride (KCl), magnesium sulfate heptahydrate ($MgSO_4 * 7\ H_2O$), sodium carbonate (Na_2CO_3), copper(II) sulfate pentahydrate ($CuSO_4 * 5\ H_2O$) and *o*-nitrophenyl-β-galactopyranoside (ONPG) were purchased from Sigma Aldrich (St. Louis, MO, USA), Fluka (Buch, Switzerland), and Merck (Darmstadt, Germany). Cucurbitin was purchased from Phytolab (Vestenbergsgreuth, Germany).

For yeast media preparation, yeast nitrogen base was purchased from Difco (Franklin Lakes, NJ), amino acids from Serva Feinbiochemica (Heidelberg, Germany), and dropout medium without tryptophan from Sigma Aldrich. Cell culture media and reagents were purchased from Biochrom (Berlin, Germany), Sigma-Aldrich or Invitrogen (Lofer, Austria).

Cell culture reagents were purchased from Biochrom (Berlin, Germany), Sigma-Aldrich (St. Louis, MO, USA) or Invitrogen (Lofer, Austria). Sodium selenite, human transferrin, methylthiazolyldiphenyl-tetrazolium bromide (MTT), staurosporine and insulin (solution from bovine pancreas) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extracts

The pumpkin seed extracts were kindly provided by APOMEDICA Pharmazeutische Produkte GmbH (Graz, Austria). The crude extract is a hydroethanolic extract (60%) of *Cucurbita pepo* L. ssp. *pepo* var. *styriaca* semen with a drug:extract ratio of 15–25:1. The content of cucurbitin measured by GC was 0.41% (m/m).

A second batch of the extract was granulated on maltodextrin (30%) as carrier.

The crude extract was diluted with distilled water (sterile filtered, 0.22 μm filters from Millipore (Millipore Ireland Ltd.) or with DMSO in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5) and 1:10 (KRD10 or KRW10). The extract granulate was extracted with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), 100 mg/ml (KGD100 or KGW100), 50 mg/ml (KGD50 or KGW50), 20 mg/ml (KGD20 or KGW20) and 10 mg/ml (KGD10 or KGW10).

2.3. Cell lines

Du-145 was obtained by ATCC (Wesel, Germany). LnCaP and BPH-1 were purchased by DSMZ (Braunschweig, Germany). Caco-2 and MCF-7 were kindly provided by Manfred Schuster (Apeiron Biologics, Vienna, Austria). HDF-5 was provided by the Cell culture working group of the department of biotechnology (Vienna, Austria).

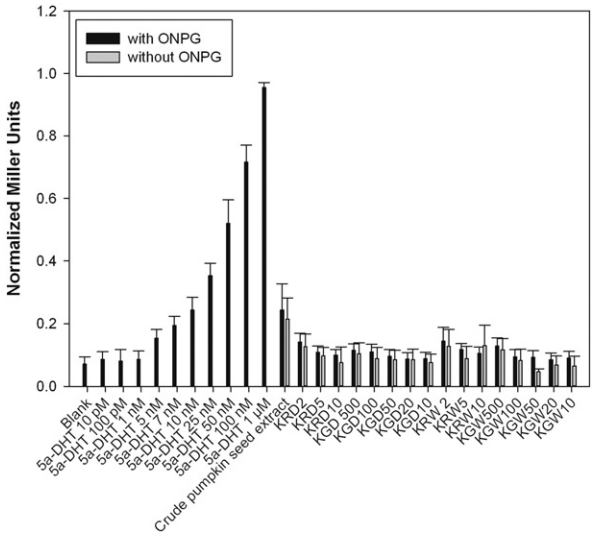

Fig. 2. Crude pumpkin seed extract and extracts of the granulated extract in DMSO and water in yeast androgen assay. 5 alpha-Dihydrotestosterone (5a-DHT) was the reference compound and DMSO was used as negative control. Extracts were tested with and without ONPG as substrate. Sample code: K..pumpkin seed, R..crude extract, G..granulate (pumpkin seed extract on maltodextrin), D..extract or dilution with DMSO, W..extract or dilution with destilled water. Crude extract was tested undiluted and in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5) and 1:10 (KRD10 or KRW10). The extract granulate was tested after extraction with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), 100 mg/ml (KGD100 or KGW100), 50 mg/ml (KGD50 or KGW50), 20 mg/ml (KGD20 or KGW20) and 10 mg/ml (KGD10 or KGW10). Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

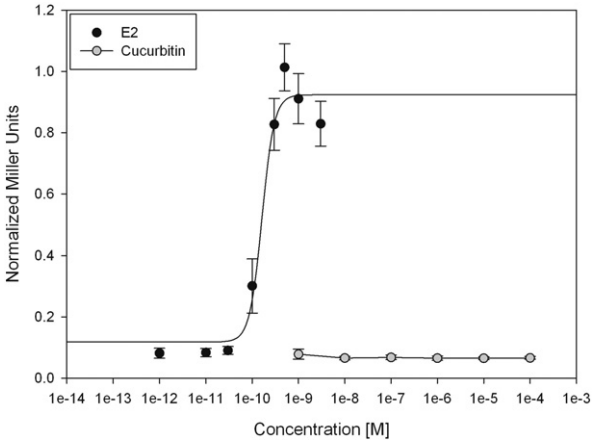

Fig. 3. Cucurbitin in yeast estrogen receptor alpha assay. 17β-Estradiol (E2) was the reference compound and DMSO was used as negative control. Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

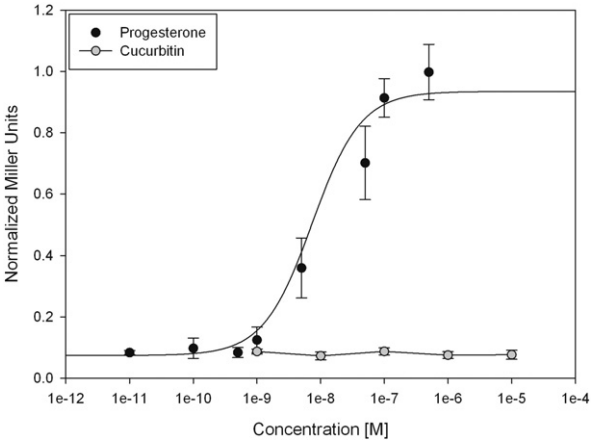

Fig. 5. Cucurbitin in yeast progesteron receptor assay. Progesterone was the reference compound and DMSO was used as negative control. Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

2.4. Transactivation assays with yeast

Yeast assay performance and evaluation of the assays with the human androgen receptor (yAS), estrogen receptor α (yESα) and progesterone receptor (yPR) have been previously described by Reiter et al. [22]. They are two-plasmid systems containing expression plasmids with the appropriate human receptor gene (AR, ERα or PR) and a LacZ reporter plasmid. The expression of the expression plasmid is induced upon addition of copper. If an agonist of the appropriate receptor is present, the hormone receptor is activated and binds as homodimer to a hormone response element (HRE) on the reporter plasmid. The gene product of the reporter plasmid is β-galactosidase, which is quantified and measured for the agonistic effect of the ligand. The specific enzyme activity, expressed in Miller Units (MU), is the β-galactosidase activity normalized to the optical density at a wavelength of 600 nm. For the evaluation, the MU was plotted against the concentration (logarithmic scaling) and the resulting curve fitted using a logistic dose response function. The calculation and fitting was performed using Table Curve 2D software (Jandel Scientific) and plotted with SigmaPlot 10.0 (Systat Software). Miller Units were normalized to the maximum of the reference compound (sum of parameters a and b of the logistic response curve, which represent the baseline and the plateau of the curve; set to 100%).

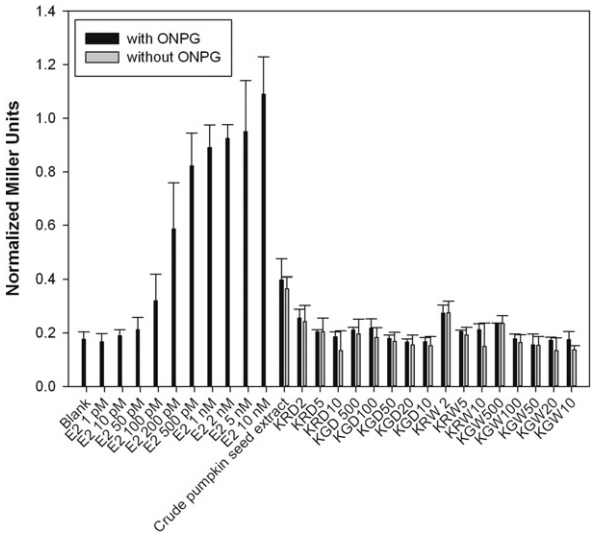

Fig. 4. Crude pumpkin seed extract and extracts of the granulated extract in DMSO and water in yeast estrogen receptor alpha assay. 17β-Estradiol (E2) was the reference compound and DMSO was used as negative control. Extracts were tested with and without ONPG as substrate. Sample code: K..pumpkin seed, R..crude extract, G..granulate (pumpkin seed extract on maltodextrin), D..extract or dilution with DMSO, W..extract or dilution with destilled water. Crude extract was tested undiluted and in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5) and 1:10 (KRD10 or KRW10). The extract granulate was tested after extraction with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), 100 mg/ml (KGD100 or KGW100), 50 mg/ml (KGD50 or KGW50), 20 mg/ml (KGD20 or KGW20) and 10 mg/ml (KGD10 or KGW10). Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

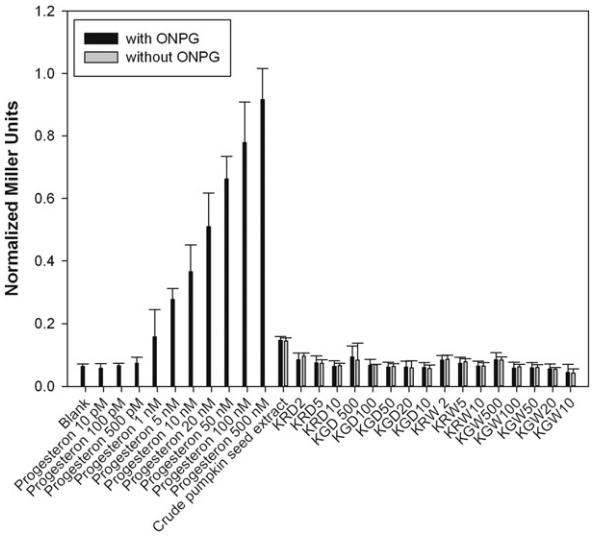

Fig. 6. Crude pumpkin seed extract and extracts of the granulated extract in DMSO and water in yeast progesterone receptor assay. Progesteron was the reference compound and DMSO was used as negative control. Extracts were tested with and without ONPG as substrate. Sample code: K..pumpkin seed, R..crude extract, G..granulate (pumpkin seed extract on maltodextrin), D..extract or dilution with DMSO, W..extract or dilution with destilled water. Crude extract was tested undiluted and in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5) and 1:10 (KRD10 or KRW10). The extract granulate was tested after extraction with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), 100 mg/ml (KGD100 or KGW100), 50 mg/ml (KGD50 or KGW50), 20 mg/ml (KGD20 or KGW20) and 10 mg/ml (KGD10 or KGW10). Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

2.5. Cell culture

Human prostate cancer cell lines LNCaP (androgen sensitive) and DU145 (androgen insensitive), human breast cancer cell line MCF-7 (ERα positive) human epithelial colorectal adenocarcinoma cell line Caco-2, human benign prostate hyperplasia cell line BPH-1 and human dermal fibroblasts were cultured in appropriate media (LNCaP: RPMI 1640 w/o phenolred +10% FCS; DU145: RPMI 1640 + 10% FCS; MCF-7: MEM Earl's Salt +2 mM *L*-alanyl glutamine, 1 mM sodium-pyruvate, 1× non-essential amino acid solution, 0.01 mg/ml bovine insulin and 10% FCS; Caco-2: MEM Earl's Salt +2 mM *L*-glutamine, 1 mM sodium-pyruvate, 1× non-essential amino acid solution and 20% FCS; BPH-1: RPMI 1640 + 5 μg/ml transferrin, 5 ng/ml sodium selenite and 5 μg/ml insulin) and incubated at 37 °C and 5% CO_2 and humidified atmosphere. Media were replaced every 2–3 days, depending on growth rate.

2.6. Cell viability assay (MTT assay)

Cells were trypsinized, harvested and diluted to a final concentration of 10^5 cells/ml. 100 μl cell suspension were applied per well (96 well plates, sterile, transparent, flat bottom, from Nunc (Roskilde, Denmark)) and incubated at 37 °C and 5% CO_2 for 24 h. Media were removed and 198 μl of fresh media were added to the adhered cells. 2 μl of sample were applied in each well. Staurosporine (end concentration of 10 μM) that induces apoptosis was used as positive control. Cells only with DMSO as vehicle in media are the reference. Each test set-up consisted of three microtiter plates. After 24 h 20 μL MTT-solution (5 g/L methylthiazolyldiphenyl-tetrazolium bromide in PBS) were added to each well of the first plate, incubated for 2 h at 37 °C and the media was removed. 100 μL DMSO was added to each well and mixed for 5 min. The absorbance of the dissolved purple formazan complex that is a measure of cell growth and proliferation activity was measured with a Tecan Genios Pro plate reader at

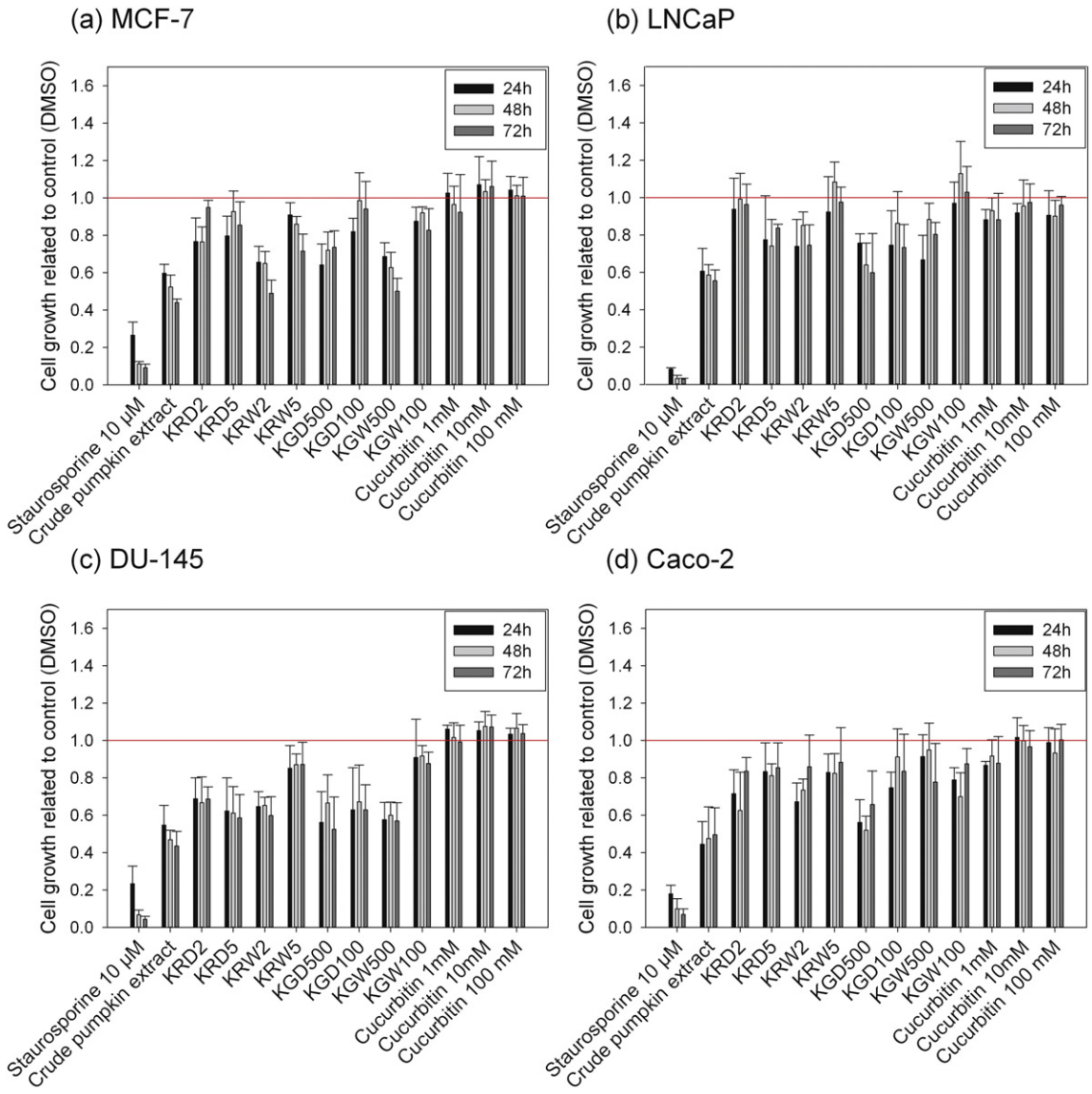

Fig. 7. Effects of hydroalcoholic pumpkin seed extracts and cucurbitin on growth of cancer cell lines. (a) Estrogen receptor positive breast cancer cell line MCF-7, (b) androgen receptor sensitive prostate cancer cell line LNCaP, (c) androgen receptor in sensitive prostate cancer cell line DU-145, and (d) colon cancer cell line (Caco-2). Staurosporine was tested as positive control. Sample code: K..pumpkin seed, R..crude extract, G..granulate (pumpkin seed extract on maltodextrin), D..extract or dilution with DMSO, W..extract or dilution with distilled water. Crude extract was tested undiluted and in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5). The extract granulate was tested after extraction with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), and 100 mg/ml (KGD100 or KGW100). Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

570 nm with a reference wavelength of 690 nm. After 48 h, the procedure was repeated with the second microtiter plate of the test run and after 72 h with the third microtiter plate. Results were related to absorbance of untreated cells (reference).

The relative growth inhibition was calculated as:

$$Cellgrowthrelated\ to\ control = \frac{Absorbance\ of\ extract\ of\ cells\ with\ treatment}{Absorbance\ of\ extract\ of\ cells\ without\ \ treatment}.$$

To evaluate the sensitivity index, cell viability assays with HDF-5 treated with crude pumpkin seed extract were conducted with varying end concentrations of extract (100 μg/mL, 150 μg/mL, 200 μg/mL, 250 μg/mL, 350 μg/mL, 400 μg/mL and 500 μg/mL).

3. Results and discussion

3.1. Androgen receptor yeast assay (yAS)

Curcubitin, a lead compound in hydroalcoholic extracts, did not transactivate the androgen receptor (Fig. 1).

The crude pumpkin seed extract and low dilutions (KRW2 and KRD2) thereof and high concentrations of the granulated extract (KGD500 and KGW500), showed a low signal above the blank. The signal was in the same range as 5-alpha-dihydrotestosterone at 5 nM final concentration (Fig. 2). But as the pumpkin extracts have a relatively strong intrinsic colour, an experiment was set up, where extracts and dilutions were tested in parallel with and without ONPG as substrate, to eliminate false-positive results. The normal yeast assay set-up is so designed that upon transactivation of the receptor, a reporter plasmid is expressed with β-galactosidase as gene product. This enzyme hydrolyses the substrate ONPG into among others ortho-nitrophenol, which gives a yellow colour. When no ONPG is present and still a signal is detected at the wavelength that is measured for the test, it is a false positive result due to intrinsic coloration of the sample. This is true for pumpkin seed extract; with and without ONPG as substrate, the same signal is detected (Fig. 2).

3.2. Estrogen receptor α yeast assay (yESα)

Curcubitin showed also no estrogenic activity in yeast assay (Fig. 3).

Similar as in the yeast androgen receptor assay, pumpkin seed extracts showed in high concentrations or in low dilutions of the crude extract, low signal in the yeast estrogen α receptor assay. This signal is in the range of 0.172 nM 17β-estradiol, which was the reference compound of the assay. Nevertheless, like in the androgen receptor assay, this signal is due to a false-positive result arising from the strong intrinsic colour of the extracts (Fig. 4).

3.3. Progesterone receptor yeast assay (yPR)

Curcubitin showed also no activity in the yeast assay with progesterone receptor (Fig. 5).

The same results were obtained as in yAS and yESα. Pumpkin seed extracts showed in high concentrations or in low dilutions of the crude extract, low signalling in yPR (Fig. 6), but the experiment with and without substrate showed that this is a false-positive result.

3.4. Cell viability assays

Crude pumpkin seed extract inhibited the cell growth in all cancer cell lines, being both prostate cancer cell lines, the androgen-sensitive LNCaP and the androgen-insensitive DU-145, the estrogen-receptor positive breast cancer cell line MCF-7 and the colorectal adenocarcinoma cell line Caco-2 (Fig. 7). This effect was not due to cucurbitin, as this compound showed no growth inhibiting effect on cancer cell lines (Fig. 7).

The growth inhibition was also observed in the hyperplastic, but non-carcinogenic cell line BPH-1, which stems from benign prostate hyperplasia tissue (Fig. 8). For cancer cell lines and BPH-1, a cell growth inhibition of about 40–50% was observed. The cell growth inhibiting effect was less in the human fibroblast cell line HDF-5, where only an inhibition of ~20% was observed (Fig. 8).

To investigate the sensitivity index of pumpkin seed extract, HDF-5 were treated with varying end concentrations of crude pumpkin seed

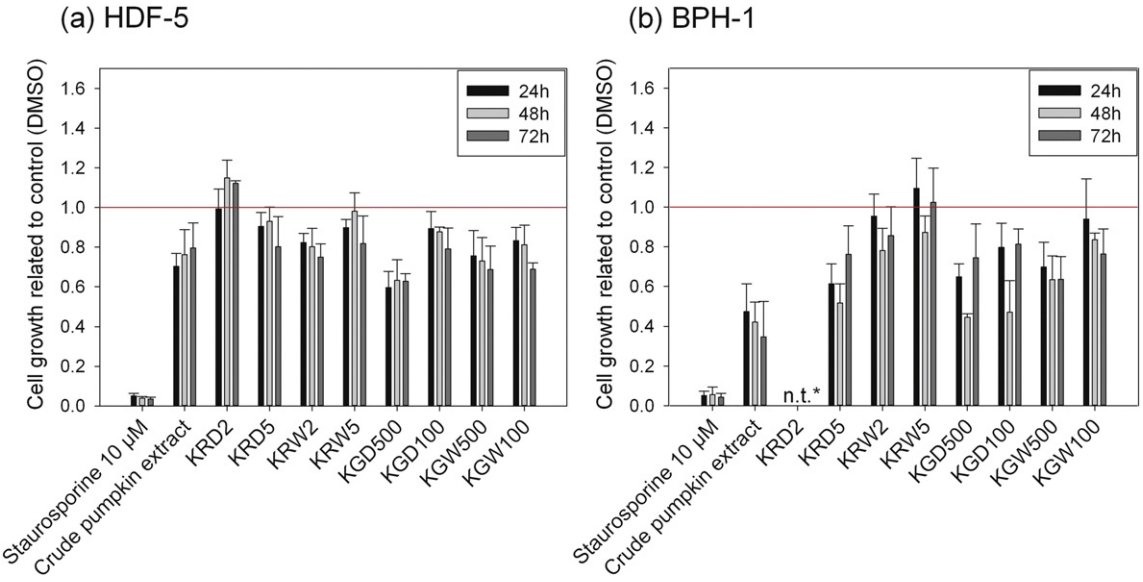

Fig. 8. Effects of hydroalcoholic pumpkin seed extracts on growth of non-transformed human dermal fibroblasts (HDF-5) (a) and human benign prostate hyperplasia cell line (BPH-1) (b). Staurosporine was tested as positive control. Sample code: K..pumpkin seed, R..crude extract, G..granulate (pumpkin seed extract on maltodextrin), D..extract or dilution with DMSO, W..extract or dilution with distilled water. Crude extract was tested undiluted and in dilutions 1:2 (KRD2 or KRW2), 1:5 (KRD5 or KRW5). The extract granulate was tested after extraction with DMSO or water in concentrations of 500 mg/ml (KGD500 or KGW500), and 100 mg/ml (KGD100 or KGW100). Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates. Abbreviations: n.t., not tested.

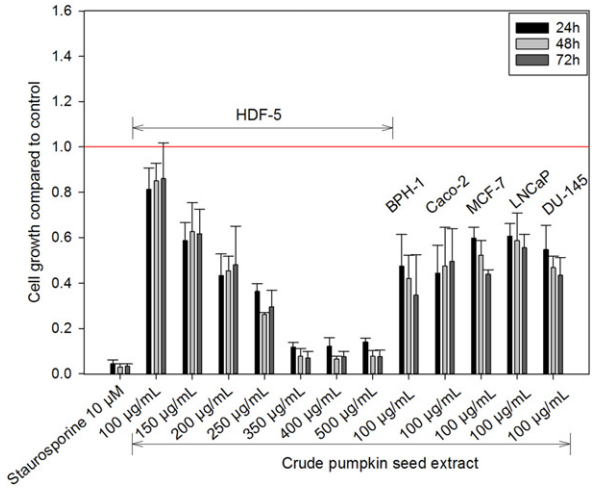

Fig. 9. Comparison of growth inhibition of crude pumpkin seed extract between non-transformed human dermal fibroblasts (HDF-5 cell line), human benign prostate hyperplasia cell line (BPH-1) and cancer cell lines (human prostate cancer cell line LNCaP (androgen sensitive), DU-145 (androgen insensitive), human breast cancer cell line MCF-7 (ERalpha positive) and human epithelial colorectal adenocarcinoma cell line Caco-2). Staurosporine was tested as positive control. Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

extract (100 μg/mL, 150 μg/mL, 200 μg/mL, 250 μg/mL, 350 μg/mL, 400 μg/mL and 500 μg/mL) and compared to the effect of 100 μg/mL crude pumpkin seed extract on all other cell lines. A dose-dependent decrease of cell viability can be observed, with IC_{50} values (mean ± S.E.M) of 177.05 ± 38.22 μg/mL after 24 h, 189.41 ± 11.44 μg/mL after 48 h and 192.22 ± 18.51 after 72 h. In direct comparison, it can be seen that a 1.5 to 2fold concentration of the extract is necessary to elicit the same effect on HDF-5 as on the cancer cell lines or BPH-1 (Fig. 9).

Testing three different batches of crude pumpkin seed extracts did not show a variation. Only one cell line (BPH-1) was used for this purpose (Fig. 10).

4. Conclusions

Ethnomedicine represents a treasure trove for modern medicine and pharmaceutical industry. The oral traditions and the experience of healers that have been collected over the centuries have great potential. But modern research has to investigate if ethnomedicinal records have a scientific basis.

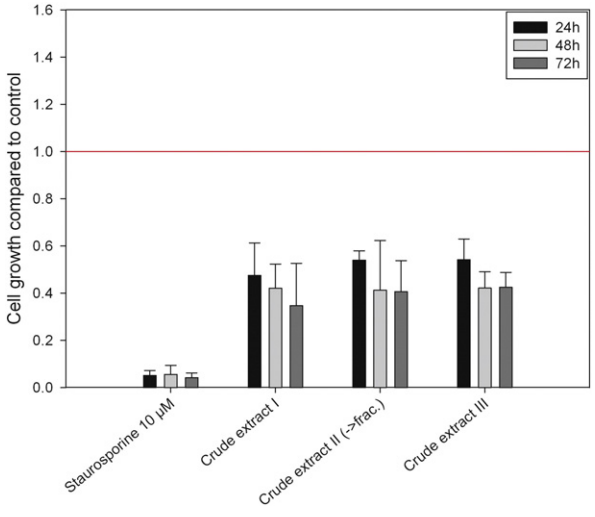

Fig. 10. Testing of batch to batch variability of crude pumpkin extraction on growth inhibition of human benign prostate hyperplasia cell line (BPH-1). Staurosporine was tested as positive control. Mean values ± SD are given. Experiments were performed in at least three independent assays and within these tests in at least duplicates.

Pumpkin is a vegetable that is consumed all over the world as food. But especially pumpkin seeds have also been used in traditional medicine worldwide. Unfortunately, studies that investigate this plant material are scarce and data are insufficient to draw a definite conclusion about the efficacy of pumpkin seeds for the treatment or prevention of diseases.

Pumpkin seeds are used in Central Europe as remedy for benign prostate hyperplasia and bladder problems. Especially in Austria, a special variety is well known. The Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca*) is unique among pumpkins as the seeds of this variety have no outer shell due to a mutation. The famous Styrian pumpkin seed oil is produced from these seeds after roasting. But not only the oil, also have hydroethanolic extracts been associated with beneficial health effects. Our studies clearly show that these effects are not mediated through sex steroid hormone receptors. Prostate hyperplasia has been associated with aberration in regulation of steroid hormone receptors. Thus it is obvious to test if these extracts are able to modulate steroid hormone pathway. Hydroalcoholic pumpkin seed extract inhibits growth of cancer cells but also hyperplastic cells, while its effects on non-hyperplastic cells are much weaker. This effect is of high significance. Every compound that is able to be more effective against hyperplastic cells such as cancer cells or fast growing cells found for example in prostatic hyperplasia tissue, and be only moderate effective against non-hyperplastic cells, provides new therapeutic opportunities. Hence, if our *in vitro* results were to be mirrored *in vivo*, pumpkin extract would be truly an interesting tool at least as accompanying remedy for example for benign prostate hyperplasia, a common disease in men over fifty years of age. This *in vitro* evidence also corroborates the observation in the clinical study of Friedrich at al. [14] and experience in ethnomedicine. The extract used in this study shows a high drug:extract ratio of 15–25:1, hence we consider it preferable to use an extract instead of crude plant material to get rational and relevant concentrations.

Our study indicates that the effect is not mediated by cucurbitin, the lead compound used for standardisation of such extracts. We also show that different batches of pumpkin seed extract have the same *in vitro* properties in cell culture. Although the compound which is used to standardize the extract is not our active ingredient for the effects that we observed, we can conclude that the standardized production of this extract is nevertheless efficient. In this case the often criticised shortcoming of plant extracts, namely being poorly standardized, has been overcome. In general, the active ingredients of phytopharmaceuticals as used in ethnomedicine are mostly unknown and the plant extract as such is considered as active ingredient. Often it is a mixture of active compounds and their concerted action that mediates pharmaceutical activity. Nevertheless, the isolation of active compounds should be the next step in order to elucidate the phytochemical basis of the growth inhibitory effects observed in the current study.

Conflict of interest

The study was in part sponsored by the Christian-Doppler-Laboratory of Receptor Biotechnology (70%) and by APOMEDICA Pharmaceutical Products GmbH (30%). These funding sources had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the manuscript and in the decision to submit the article for publication. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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