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(54) Title: THE USE OF EXTRACTS OR MATERIALS EXTRACTED FROM PIPER CUBEBA L. AS AN EFFECTIVE COMPONENT IN A DRUG FOR THE TREATMENT OF CANCER DISEASES

(54) Bezeichnung: VERWENDUNG VON EXTRAKTEN ODER EXTRAKTIVSTOFFEN AUS PIPER CUBEBA L. ALS WIRKSAME BESTANDTEILE IN EINEM MEDIKAMENT ZUR BEHANDLUNG VON KREBSERKRANKUNGEN

(57) Abstract: The present invention relates to a method for the preparation of a dry extract from the fruit of Piper cubeba L., which characterized in that, in a first step, the fruit of Piper cubeba L. either is subjected to a steam distillation for the removal of essential oils and the distillate is removed or extracted at least once with a lipophilic phase and the lipophilic extract or extracts are removed; in a second step, the fruit, so treated, is extracted at least once either with at least one alcohol or with a mixture of at least one alcohol and water; and, in a third step, the extracted fruit parts are removed and an auxiliary material is added to the extract so obtained, which is then concentrated to a thick extract with an alcohol concentration of 0.1 to 10% (w/w), then dried and recovered.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung eines Trockenextraktes aus Früchten von Piper cubeba L., welches dadurch gekennzeichnet ist, dass man in einem ersten Schritt die Früchte von Piper cubeba L. zur Entfernung der ätherischen Öle entweder einer Wasserdampfdestillation unterzieht und das Destillat entfernt oder wenigstens einmal mit einer lipophilen Phase extrahiert und diesen lipophilen Extrakt oder diese lipophilen Extrakte entfernt; in einem zweiten Schritt die so behandelten Früchte wenigstens einmal entweder mit wenigstens einem Alkohol oder mit einem Gemisch aus wenigstens einem Alkohol und Wasser extrahiert; und in einem dritten Schritt die extrahierten Fruchtteile entfernt, den so erhaltenen Extrakt nach der Zugabe eines Hilfsstoffes zuerst auf eine Konzentration an Alkohol zwischen 0,1 und 10 m/m % zu einem Spissumextrakt konzentriert, dann trocknet und gewinnt.

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Use of extracts or of extract compounds from Piper cubeba L. as active components in a medicament for the treatment of cancer

The present invention is directed to the use of extracts or of extract compounds from Piper cubeba L. as active components in a medicament for the treatment of cancer.

Uses of Piper cubeba L. compositions are described for example in Hunnius, Pharmazeutisches Wörterbuch, 8th edition, 1998, pages 1084 to 1085.

Herein are described popular applications, such as the treatment of headache as well as the use as diuretic, urine disinfectant and stomachic.

As drug is used the immature fruit, from which also the cubeben-oil, the essential oil of the cubeben, is obtained by steam distillation.

Oleum Cubebae is applied in the same indications as the fruits.

According to J. Seidemann in "World Spice Plants", Springer-Verlag, 2005, page 291 Piper cubeba L. is used for the aromatization of liqueur, ginger bread, and honey bread. As product is used the essential oil that is obtainable from the immature fruit.

In JP 2000-095649 A are described extracts, among others also from the cubeben fruit that are obtained by means of a hydrophilic solvent, for example acetone, methanol and ethanol, or their mixtures with

water. Thus, such extracts contain both the essential oil and hydrophilic substances.

These extracts shall act as testosteron-5α-reductase inhibitors. Thereby these extracts shall influence positively the growth of hair.

These extracts shall also serve for the treatment of benign prostate hyperplasia.

In this document are given in table 1 IC_{50}-values that are referred to the inhibition of the testosteron-5α-reductase.

The extract of cubeben inhibits according to this table the enzyme for 50% at a concentration of 0.79 mg/ml.

A. Chatterjee et.al., Jour. Indian Chem. Soc., Vol. 45, No. 8, 1968, pages 723 to 725 describes the spectral characteristics of the pure substance Cubebin. This chemical compound was isolated from an alcoholic extract of the defatted fruits of Piper cubeba L..

A. Dasgupta et.al., Quart. J. Crude Drug Res., 18, (1980), No. 1, pages 17 to 25 describes the use of the essential oil of Piper cubeba L. for the treatment of for example cystitis, and gonorrhoea.

Eun-Mi Choi et. al., Journal of Ethnopharmacology, Elsevier Scientific Publishers Ltd., 89, 2003, pages 171 to 175 describes anti-inflammatory characteristics of an extract prepared with 80% methanol from dried fruits of Piper cubeba L..

During a screening-process were tested several tropical medicinal plants upon their activity against tumor cells *in vitro*.

It was found quite surprisingly that an ethanolic extract from immature fruits of cubeben kills all tested tumor cells.

As predominantly the essential oil of the cubeben fruits is used in medicine it was obvious to extract the fruits with a suitable extracting agent in order to obtain the essential oil.

This was realized by an exhausting extraction with hexane.

The fruits, exempted in this way from the essential oil, were for the sake of completeness extracted additionally with 90% aqueous ethanol in order to obtain medium polar extract compounds.

Both the obtained essential oil and the ethanolic secondary extract were then tested upon their activity against tumor cells.

As expected it was found that the obtained essential oil killed all tested tumor cells. This points to the fact that the observed cytotoxic effect is of unspecific nature and thus demonstrates no antitumor effect.

But quite surprisingly it was found that the ethanolic secondary extract indeed did not kill directly any of the tested tumor cells but changed in some tumor cells their proliferation behaviour.

Such tumor cells proved to be as especially sensitive against the ethanolic secondary extract that need for their growth sex hormones as growth factors. As examples are mentioned the breast cancer cell line MCF 7 and the prostate cancer cell line LnCAP. This observation allows the conclusion that the proliferation inhibiting activity may not rely primary on an inhibition of the testosteron-5α-reductase as this is irrelevant for the growth of the breast cancer cell line MCF 7.

This invention relates to a process for the preparation of an extract from fruits of Piper cubeba L.

This extract shall be free or nearly free from cytotoxic essential oils.

This extract shall inhibit the growth especially of such tumor cells that need for their growth sex hormones as growth factors.

This extract shall show anti-androgenic and/or anti-estrogenic activities.

This extract shall antagonize the activities of the sex hormone dihydrotestosterone, abbreviated with DHT, especially its proliferation enhancing and anti-apoptotic effect on prostate cancer cells.

The invention is characterized by the characteristics as defined in the independent claims.

In an embodiment the invention provides a process for the preparation of a dry extract of fruit of Piper cubeba L. wherein

- in a first step for the removal of the essential oils the fruit of Piper cubeba L. either

-- are subjected to a steam distillation and the distillate is removed, or

-- are extracted at least once with a lipophilic phase and this lipophilic extract or these lipophilic extracts is (are) removed,

- in a second step the so-treated fruit is extracted at least once either with at least one alcohol or with a mixture of at least one alcohol and water, and

- in a third step the extracted fruit parts are removed, the so-obtained extract after the addition of an auxiliary agent is first concentrated to a concentration of alcohol between 0.1 and 10 m/m % to a spissum extract, is then dried and obtained.

In an embodiment the invention provides use of extracts or of extract compounds from Piper cubeba L., whereby these extracts or these extract compounds have anti-androgenic and/or anti-estrogenic activities, as active components in a medicament for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases.

In an embodiment the invention provides use of an extract that has been obtained according to the process according to the invention as active component in a medicament for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases, and benign prostate hyperplasia.

In an embodiment the invention provides use of an extract that has been obtained according to the process according to the invention, whereby this extract antagonize the activities of the sex hormone dihydrotestosterone, abbreviated with DHT, especially its proliferation enhancing and anti-apoptotic activity on prostate cancer cells, as active component in a medicament for the treatment of prostate cancer, including its metastases, and of benign prostate hyperplasia.

In an embodiment the invention provides medicament when used for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases, and of benign prostate hyperplasia, characterized in that it contains an extract or extract compounds from Piper cubeba L. as active components, whereby this extract or these extract compounds have anti-androgenic and/or anti-estrogenic activities.

In an embodiment the invention provides use of an extract that has been obtained according to the process according to the invention, whereby this extract antagonize the activities of the sex hormone dihydrotestosterone, abbreviated with DHT, especially its proliferation enhancing and anti-apoptotic effect on prostate cancer cells, for the preparation of a medicament for the treatment of prostate cancer, including its metastases, or of benign prostate hyperplasia.

Preferred embodiments are defined in the dependent claims.

In the following part are described possible embodiments of the present invention.

Thereby is made also reference to the figures.

Figure 1a shows the anti-proliferate effect of the extract prepared according to example 1 on LNCap and PC-3 cells.

Figure 1b shows the anti-proliferate effect of the pure substance Cubebin on LNCap and PC-3 cells.

Figure 2 shows the inhibition of the DNA-synthesis of LNCap cells with the extract prepared according to example 1.

Figure 3 shows the anti-androgenic effect of the extract prepared according to example 1 on the androgen-dependent cell proliferation on LNCap cells.

Figure 4 shows the anti-androgenic effect of the extract prepared according to example 1 on the DNA-synthesis of LNCap cells.

Figure 5 shows the anti-estrogenic effect of the extract prepared according to example 1 on the DNA-synthesis of MCF-7 cells.

Figure 6a shows the inhibiting effect of the extract prepared according to example 1 and of the pure substance Cubebin on the activity of 5α-reductase type II.

Figure 6b shows the inhibiting effect of the known 5α-reductase inhibitor "Finasterid" on the activity of 5α-reductase type II.

Figure 7a shows that TNF-α induces the apoptosis of the tumor cells in dependency of the dose, and that this effect is abolished completely with DHT in the tumor cells.

Figure 7b shows that the anti-apoptotic effect of DHT is abolished by the extract prepared according to example 1.

Figure 8 shows that both the extract prepared according to example 1 and the pure substance Cubebin inhibit the secretion of the prostate specific antigen (PSA) in dependency of the respective dose.

Figure 9 shows that the extract prepared according to example 1 inhibits strongly the with DHT induced secretion of the prostate specific antigen (PSA).

Figure 10 shows that the androgen receptor concentration in LNCap cells is reduced increasingly in dependency of the dose both by the treatment with the extract prepared according to example 1 and by the treatment with the pure substance Cubebin.

The following examples illustrate the present invention.

Example 1 (Preparation of a liquid extract)

110 g of immature, dried fruits of Piper cubeba L. having a grinding fineness from 0.1 mm to 0.9 mm

were extracted under stirring at a temperature between 10°C and 20°C during 8 hours with 0.5 liters of hexane. The hexane layer charged with the essential oils and highly lipophilic components was then separated. This procedure was carried out once more, whereby the extraction time was limited to 2 hours.

The so defatted fruits were then dried in a vacuum cabinet at a temperature of 40°C until constancy of the weight. There were obtained 92 g of defatted drug material.

Then the so treated fruits were extracted under stirring at a temperature between 20°C and 30°C during 2 hours with a mixture of 90 parts by weight of ethanol and 10 parts by weight of water.

The weight ratio between the drug and the extraction mixture was 1:5.

The so extracted drug was separated by means of layer filtration. There were obtained 380 g of a dark brown liquid extract having a dry substance content of 1.92 m/m %, corresponding to a yield of extract compounds of 7.3 g absolute from 92 g defatted fruits.

This extract is denoted in the following part as P9605.

This extract contains 20 m/m % of Cubebin, referred to the dry substance content.

Example 2 (Preparation of a dry extract)

A liquid extract obtained according to example 1 was added dose by dose into an evaporator at a temperature of 40°C, and the evaporation was started under vacuum (300 mbar to 20 mbar) and elevated temperature (40°C to 55°C).

During the distillation the remaining part of the fluid extract was added continuously dose by dose into the evaporator until the total amount of the fluid extract has been added and until in the obtained spissum extract a dry substance content from 30 to 40 m/m % was reached.

There were obtained 20.0 g of spissum extract with a dark brown colour, free flowing and of homogenous consistency. The spissum extract showed a dry substance content of 36.5 m/m %, what corresponds to a content of extract compounds of 7.3 g.

This concentrated spissum extract was mixed homogenously with 7.8 g of an aqueous 40 m/m % gum arabic solution and then dried in a dryer under vacuum at a pressure from 150 mbar to 10 mbar and a temperature from 40°C to 55°C.

There were obtained 10.4 g of an ochre brown dry extract having a content of 30 m/m % of gum arabic as auxiliary agent.

Example 3 (Preparation of a dry extract-oil suspension)

A liquid extract obtained according to example 1 was added dose by dose into an evaporator at a temperature of 40°C, and the evaporation was started under

vacuum (300 mbar to 20 mbar) and elevated temperature (40°C to 55°C).

During the distillation the remaining part of the fluid extract was added continuously dose by dose into the evaporator until the total amount of the fluid extract has been added and until in the obtained spissum extract a dry substance content from 10 to 20 m/m % was reached.

There were obtained 54.0 g of spissum extract with a dark brown colour, free flowing and of homogenous consistency. The spissum extract showed a dry substance content of 15.7 m/m %, what corresponds to a content of extract compounds of 7.3 g.

This spissum extract of low viscosity was mixed with 6.8 g of middle chain triglycerides (Ph. Eur.) and 0.5 g of soya-lecithin (ÖAB 90) and added dose by dose into an evaporator at a temperature of 40°C. The evaporation of this mixture was carried out during such a long time under vacuum (300 mbar to 40 mbar) and at elevated temperature (40°C to 50°C) until in the obtained spissum extract a dry substance content from 70 to 80 m/m % was reached.

There was obtained a viscous spissum extract that was then dried in a dryer under vacuum at a pressure from 150 mbar to 10 mbar and a temperature from 40°C to 55°C until a dry substance content of 99.5 m/m % was reached.

There were obtained 14.9 g of a dark brown dry extract-oil suspension having a content of 49 m/m % of

middle chain triglycerides and 3.36 m/m % soya-lecithin as auxiliary agents.

Example 4 (Inhibition of the cell proliferation)

With the liquid extract P9605 prepared according to example 1 were carried out cell proliferation tests. As control was carried along a typical substance of content of the cubeben fruits, the lignan Cubebin.

For the measurement of the inhibition of the cell proliferation this extract was added to LNCap and to PC-3 cells. The so treated cells were cultivated during 4 days in 10% FBS culture medium.

For comparison the pure lignan Cubebin, that is contained in the extract prepared according to the invention and according to example 1 in an amount up to 20 m/m % of the dry substance, was added also to LNCap and to PC-3 cells. The so treated cells were cultivated during 4 days in 10% FBS culture medium.

Thereby it was proceeded according to T. Lindl, Zell- und Gewebekultur, 4th revised edition, 2000, Spektrum Akademischer Verlag, Heidelberg.

All obtained datas are given in percents with regard to the solvent control (test without extract and without Cubebin); there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

From the datas as shown in Figures 1a and 1b it is obvious that both the extract prepared according

to the invention and the pure substance Cubebin show an anti-proliferate effect on LNCap and PC-3 cells in dependency of the respective dose.

The inhibition was more pronounced on LNCap cells than on PC-3 cells.

It is obvious from Figures 1a and 1b that the inhibitive activity of the extract P9605 prepared according to example 1 is much stronger than this would be explainable by its content of Cubebin. The extract contains only 20 m/m % of Cubebin, but shows the same (LNCap) or a stronger (PC-3) inhibitive activity.

Example 5 (Inhibition of the DNA synthesis)

With the liquid extract P9605 prepared according to example 1 were carried out DNA synthesis tests.

For the measurement of the inhibition of the DNA synthesis the extract prepared according to the present invention was added to LNCap cells. The so treated cells were cultivated during 4 days in 10% FBS culture medium.

Then was measured the amount of incorporated ^{3}H-thymidin.

Thereby it was proceeded according to T. Lindl, Zell- und Gewebekultur, 4th revised edition, 2000, Spektrum Akademischer Verlag, Heidelberg.

All obtained datas are given in percents with regard to the solvent control (test without extract);

there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 2 that the extract prepared according to the present invention inhibits the DNA synthesis in dependency of the respective dose.

Example 6 (Anti-androgenic effect on the cell proliferation)

The anti-androgenic effect on the androgen dependent cell proliferation of the liquid extract P9605 prepared according to example 1 was determined.

Thereby the extract prepared according to the present invention was added to LNCap cells. The so treated cells were cultivated during 6 days in 10% CSS culture medium.

This cultivation was realized once without the addition of dihydrotestosterone, abbreviated with DHT, and once with the addition of 1 nM DHT.

Then was determined the influence of the extract prepared according to the present invention on the cell proliferation of the tumor cells on the basis of the DNA content.

All obtained datas are given in percents with regard to the solvent control (test without extract); there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 3 that the extract prepared according to the present invention overexcites in dependency of the dose the stimulating effect of DHT on the cell proliferation of the tumor cells and in addition lowers the basal proliferation of the cells.

It is known that DHT enhances the cell proliferation; see the control value at zero.

Example 7 (Anti-androgenic effect on the DNA synthesis)

The anti-androgenic effect on the DNA synthesis of the liquid extract P9605 prepared according to example 1 was determined.

Thereby the extract prepared according to the present invention was added to LNCap cells. The so treated cells were cultivated during 6 days in 10% CSS culture medium.

This cultivation was realized once without the addition of dihydrotestosterone, abbreviated with DHT, and once with the addition of 1 nM DHT.

Then was measured the amount of incorporated ^{3}H-thymidin.

Thereby it was proceeded according to T. Lindl, Zell- und Gewebekultur, 4th revised edition, 2000, Spektrum Akademischer Verlag, Heidelberg.

All obtained datas are given in percents with regard to the solvent control (test without extract);

there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 4 that the extract prepared according to the present invention overexcites in dependency of the dose the stimulating effect of DHT on the DNA synthesis of the tumor cells and in addition lowers the basal DNA synthesis of the cells.

It is known that DHT enhances the DNA synthesis; see the control value at zero.

Example 8 (Anti-estrogenic effect on the DNA synthesis of breast tumor cells)

The anti-estrogenic effect on the DNA synthesis of breast tumor cells of the liquid extract P9605 prepared according to example 1 was determined.

Thereby MCF-7 cells were cultivated during 3 days in 10% CSS culture medium to which were added different concentrations of estradiol.

This cultivation was realized once without the addition of the extract prepared according to the present invention and once with the addition of 10 µg/ml of the extract.

Then was measured the amount of incorporated ^{3}H-thymidin.

Thereby it was proceeded according to T. Lindl, Zell- und Gewebekultur, 4th revised edition, 2000, Spektrum Akademischer Verlag, Heidelberg.

All obtained results are given in DPM (radioactive degradation per minute); there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 5 that the extract prepared according to the present invention stops completely or nearly completely the stimulation of the DNA synthesis of breast cancer cells by estradiol.

It is known that estradiol enhances the DNA synthesis of breast cancer cells; see the control value at zero.

Example 9 (Inhibition of the 5α-reductase type II activity)

The inhibition of the 5α-reductase type II activity by means of the liquid extract P9605 prepared according to example 1 was determined.

The assay was realized with a homogenate of HEK293 cells that over express the 5α-reductase type II (Reichert W., Hartmann R.W. and Jose J.; 2001, Journal Enzyme Inhibition, Vol. 16, 47-53).

The influence of the extract prepared according to the present invention and of the pure substance Cubebin on the activity of the 5α-reductase type II was determined by means of the measurement of the conversion of ^{3}H-testosterone in ^{3}H-DHT.

As control substance was used the known 5α-reductase inhibitor "Finasterid".

All obtained datas are given in percents with regard to the solvent control (test without extract); there are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 6a that both the extract prepared according to the present invention and the pure substance Cubebin show an inhibitive effect on the activity of the 5α-reductase type II.

The inhibition is stronger with the extract than with the pure substance Cubebin.

The extract inhibits with an IC_{50}-value of 3.6 µg/ml, whereas the pure substance Cubebin inhibits with an IC_{50}-value of 9.9 µg/ml.

The course of the dose activity graph of the extract and of the pure substance Cubebin are analogous to the course of the dose activity graph of the known 5α-reductase inhibitor "Finasterid" (Figure 6b).

Example 10 (increase of apoptosis)

The induction of apoptosis by means of the liquid extract P9605 prepared according to example 1 was determined.

As preliminary test was added for the measurement of the induction of apoptosis the tumor necrosis factor TNF-α alone as well as in combination with 100 nM dihydrotestosterone, abbreviated with DHT, to LNCap cells. The so treated cells were cultivated during 2 days in 10% FBS culture medium.

The apoptosis of the cells was measured by applying a commercial apoptosis-immuno-assay-kit in which are detected specifically the DNA and histon fragments that are present as mono and oligonucleosomes.

It is obvious from the datas shown in Figure 7a that TNF-α induces the apoptosis of the tumor cells in dependency of the dose.

This effect is revoked completely or nearly completely with DHT in the tumor cells.

Analogous experiments were carried out with DHT alone as well as in combination with 10 µg/ml of the extract prepared according to the present invention.

It is obvious from the datas shown in Figure 7b that the anti-apoptotic activity of DHT is revoked by the extract prepared according to the present invention.

Example 11 (Inhibition of the secretion of the prostate specific antigen)

The inhibition of the prostate specific antigen (PSA) by means of the liquid extract P9605 prepared according to example 1 was determined.

Thereby in one experiment LNCap cells were cultivated during 2 days in 10% CSS culture medium to which were added different concentrations either of the extract prepared according to the present invention or of the pure substance Cubebin.

Then was measured the secreted PSA amount in the cell supernatant by means of an immuno-Assay. Additionally the amount of DNA was determined.

In Figure 8 is shown the proportion in percents of the amount of PSA to the amount of DNA.

There are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 8 that both the extract and the pure substance Cubebin inhibit the secretion of the prostate specific antigen (PSA) in dependency of the respective dose.

In a second experiment LNCap cells were cultivated during 2 days in 10% CSS culture medium to which were added different concentrations of dihydrotestosterone, abbreviated with DHT.

This cultivation was realized once without the addition of the extract prepared according to the present invention and once with the addition of 10 µg/ml of extract.

Then was measured the secreted PSA amount in the cell supernatant by means of an immuno-Assay. Additionally the amount of DNA was determined.

In Figure 9 is shown the proportion in percents of the amount of PSA to the amount of DNA.

There are given the average values with Standard deviation from 4 experiments with 3-fold repetition.

It is obvious from the datas shown in Figure 9 that the secretion of the prostate specific antigen (PSA) induced by DHT is strongly inhibited by the extract prepared according to the present invention.

Example 12 (Generation of androgen receptors)

The influence of the generation of androgen receptors by means of the liquid extract P9605 prepared according to example 1 was determined.

Thereby in one experiment LNCap cells were cultivated during 2 days in 10% FBS culture medium to which were added different concentrations either of the extract prepared according to the present invention or of the pure substance Cubebin.

Then was determined the change of the amount of the androgen receptor by means of the Westernblot analysis.

In Figure 10 are shown the bands of the androgen receptor.

The androgen receptor denseness in LNCap cells is reduced increasingly in dependency of the dose both by the treatment with the extract prepared according to the present invention and by the treatment with the pure substance Cubebin.

Conclusions

In the examples 1 to 3 is shown by which combinations of process steps extracts of cubeben fruits may be prepared that are free or nearly free of essential oil, show new characteristics and met the objects of the present invention.

Examples 4 to 12 demonstrate the antitumor activity of the extracts prepared according to the present invention and illustrate the activity mechanisms that form the basis of the activity against hormone dependent tumor cells. These examples show the high therapeutical potential of the extracts prepared according to the present invention, especially for the therapy of malign diseases which progression is influenced by female or male sex hormones.

When considering the efficacy (IC_{50}: 3.6 µg/ml) of the extracts prepared according to the present invention against the human 5α-reductase in comparison to the activity mentioned in JP 2000-095649 A (IC_{50}: 790 µg/ml) then it becomes obvious that the extracts prepared according to the present invention have an about 200-fold higher activity and thus open also for the treatment of the prostate hyperplasia quite new possibilities.

CLAIMS

1. A process for the preparation of a dry extract of fruit of Piper cubeba L. wherein

- in a first step for the removal of the essential oils the fruit of Piper cubeba L. either

-- are subjected to a steam distillation and the distillate is removed, or

-- are extracted at least once with a lipophilic phase and this lipophilic extract or these lipophilic extracts is (are) removed,

- in a second step the so-treated fruit is extracted at least once either with at least one alcohol or with a mixture of at least one alcohol and water, and

- in a third step the extracted fruit parts are removed, the so-obtained extract after the addition of an auxiliary agent is first concentrated to a concentration of alcohol between 0.1 and 10 m/m % to a spissum extract, is then dried and obtained.

2. The process according to claim 1, wherein the immature fruit of Piper cubeba L. is used, and are ground immediately before the extraction and are extracted in ground form, especially with a grinding fineness from 0.1 mm to 0.9 mm.

3. The process according to any one of claims 1 to 2, wherein in the first step as lipophilic phase is used either supercritical CO_2 or a straight or branched hydrocarbon having 4 to 9 carbon atoms, especially hexane or isopentane.

4. The process according to any one of claims 1 to 3, wherein in the first step per weight proportion of fruits

to be extracted from 1 to 20 parts by weight of lipophilic phase are used and, especially from 6 to 12 parts by weight of lipophilic phase are used and, of lipophilic phase are used.

5. The process according to one of claims 1 to 4, wherein in the first step the extraction with the lipophilic phase is realized at a temperature from 0°C to 50°C, especially from 5°C to 15°C, and during a time from 2 to 4 hours.

6. The process according to one of claims 1 to 5, wherein in the second step the alcohol is an alcohol having 1 to 5 carbon atoms, especially ethanol, and that the mixture of at least one alcohol and water consists of 50 to 90 m/m % of alcohol and 50 to 10 m/m % of water, preferably of 80 to 90 m/m % of alcohol and 20 to 10 m/m % of water, whereby ethanol is preferred.

7. The process according to any one of claims 1 to 6, wherein in the second step per weight proportion of fruits to be extracted from 1 to 20 parts by weight of at least one alcohol or of a mixture of at least one alcohol and water are used and, especially from 6 to 12 parts by weight of at least one alcohol or of a mixture of at least one alcohol and water are used.

8. The process according to any one of claims 1 to 7, wherein in the second step the extraction with at least one alcohol or with a mixture of at least one alcohol and water is realized at a temperature from 20°C to 60°C and during a time from 2 to 4 hours.

9. The process according to any one of claims 1 to 8, wherein in the third step the auxiliary agent is a drying auxiliary agent, for example mannitol, and that there is concentrated to a concentration of alcohol of 5 m/m %, and that the drying is a spray drying, a belt drying or a blade drying.

10. The process according to any one of claims 1 to 9, wherein the extract obtained in the third step is free or nearly free of α-cubebene and
β-cubebene.

11. Use of extracts or of extract compounds from Piper cubeba L., whereby these extracts or these extract compounds have anti-androgenic and/or anti-estrogenic activities, as active components in a medicament for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases.

12. Use of an extract that has been obtained according to the process according to any one of claims 1 to 10 as active component in a medicament for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases, and benign prostate hyperplasia.

2008286636 17 Sep 2013

13. Use of an extract that has been obtained according to the process according to any one of claims 1 to 10, whereby this extract antagonize the activities of the sex hormone dihydrotestosterone, abbreviated with DHT, especially its proliferation enhancing and anti-apoptotic activity on prostate cancer cells, as active component in a medicament for the treatment of prostate cancer, including its metastases, and of benign prostate hyperplasia.

14. Medicament when used for the treatment of at least one disease, selected from the group consisting of cancer diseases, especially prostate cancer, testicles cancer, breast cancer, uterus cancer, including their metastases, and of benign prostate hyperplasia, characterized in that it contains an extract or extract compounds from Piper cubeba L. as active components, whereby this extract or these extract compounds have anti-androgenic and/or anti-estrogenic activities.

15. Medicament according to claim 14, wherein the active components are contained in an extract that has been obtained according to the process according to any one of claims 1 to 10.

16. Use of an extract that has been obtained according to the process according to any one of claims 1 to 10, whereby this extract antagonize the activities of the sex hormone dihydrotestosterone, abbreviated with DHT, especially its proliferation enhancing and anti-apoptotic effect on prostate cancer cells, for the preparation of a medicament

for the treatment of prostate cancer, including its metastases, or of benign prostate hyperplasia.

Viridis Pharmaceutical Limited

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

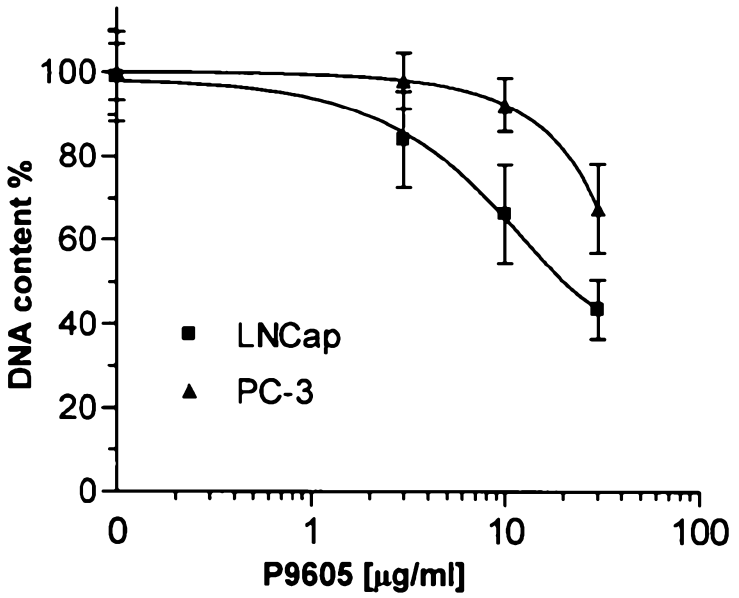
100
80
60
40
20
0
DNA content %
LNCap
PC-3
0
1
10
100
P9605 [µg/ml]

Fig. 1a

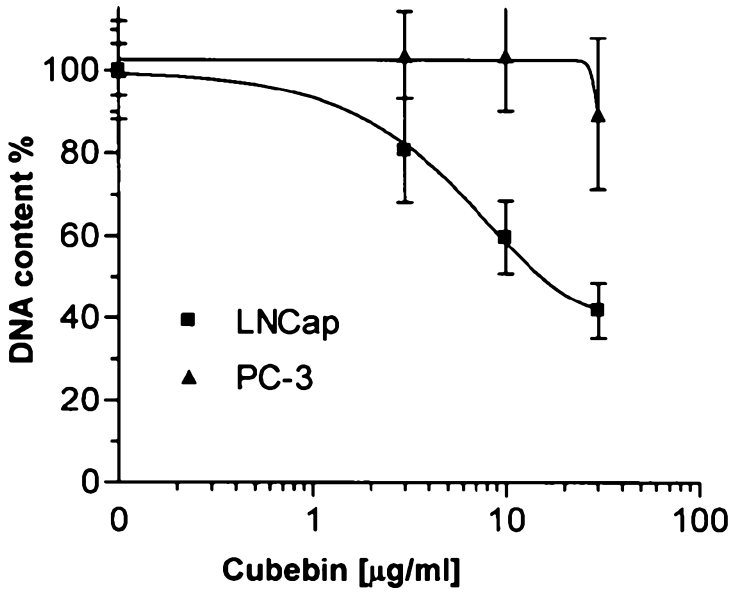
100
80
60
40
20
0
DNA content %
LNCap
PC-3
0
1
10
100
Cubebin [µg/ml]

Fig. 1b

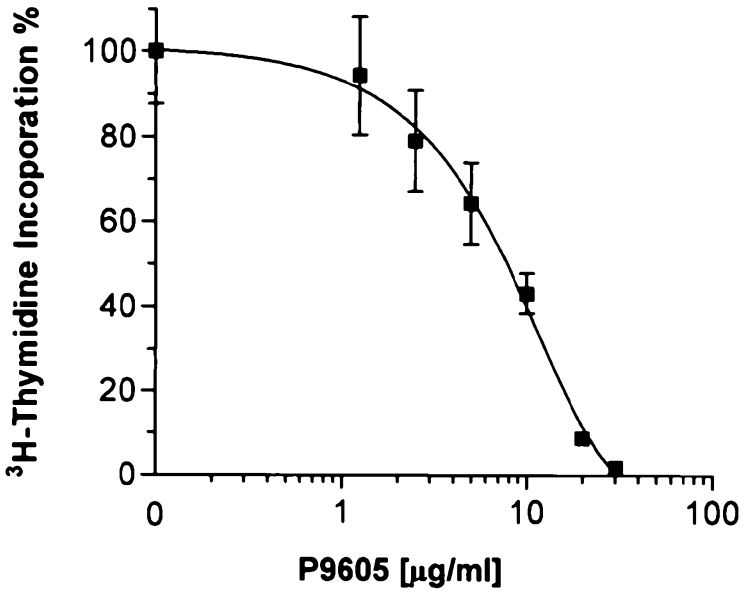
^{3}H-Thymidine Incoporation %
100
80
60
40
20
0
0
1
10
100
P9605 [µg/ml]

Fig. 2

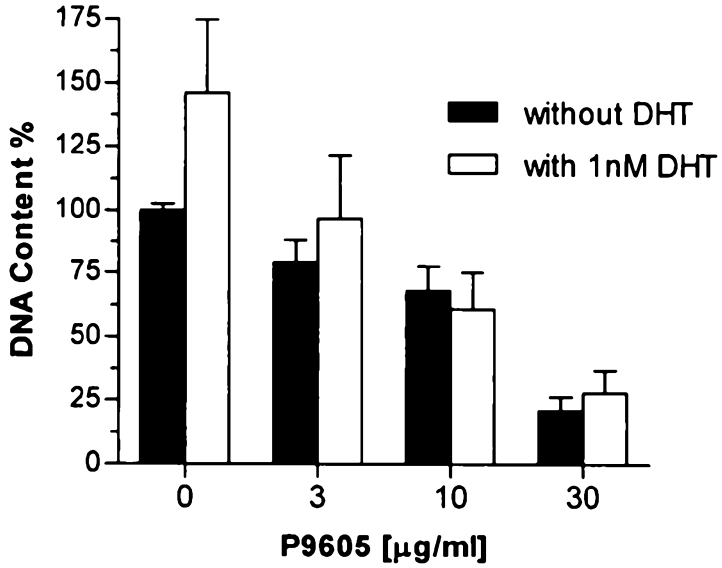
DNA Content %
175
150
125
100
75
50
25
0
0
3
10
30
P9605 [µg/ml]
without DHT
with 1nM DHT

Fig. 3

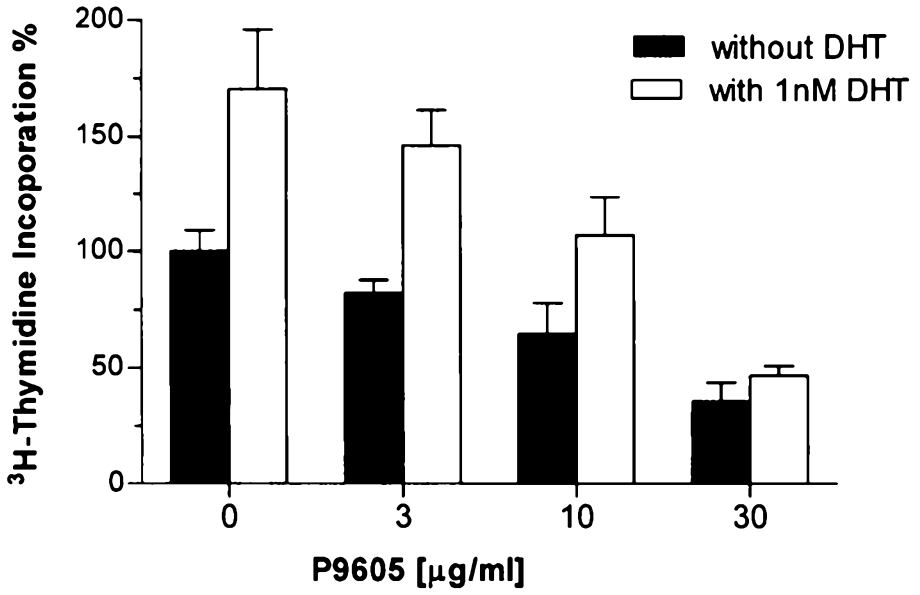
without DHT
with 1nM DHT
^{3}H-Thymidine Incoporation %
200
150
100
50
0
0
3
10
30
P9605 [µg/ml]

Fig. 4

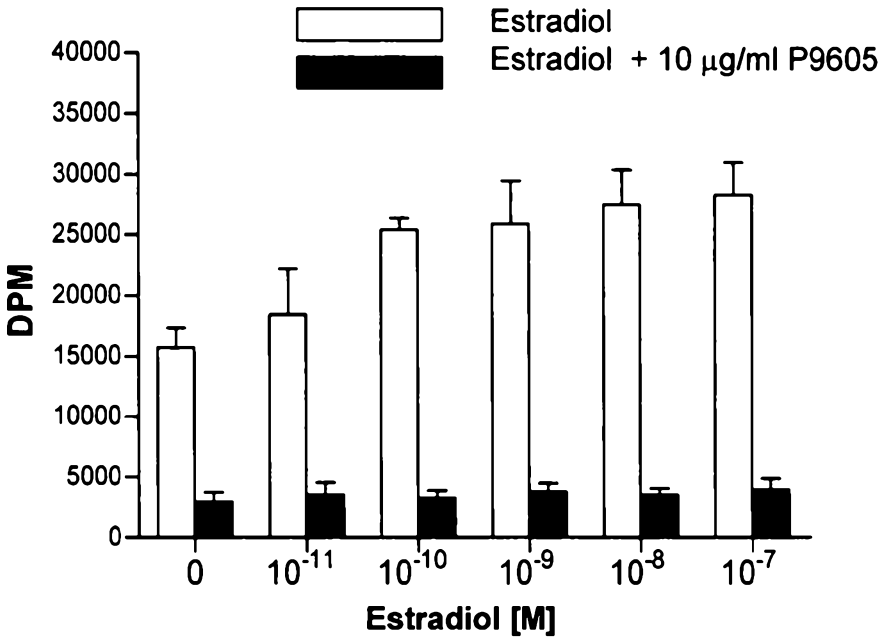
Estradiol
Estradiol + 10 µg/ml P9605
DPM
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Estradiol [M]

Fig. 5

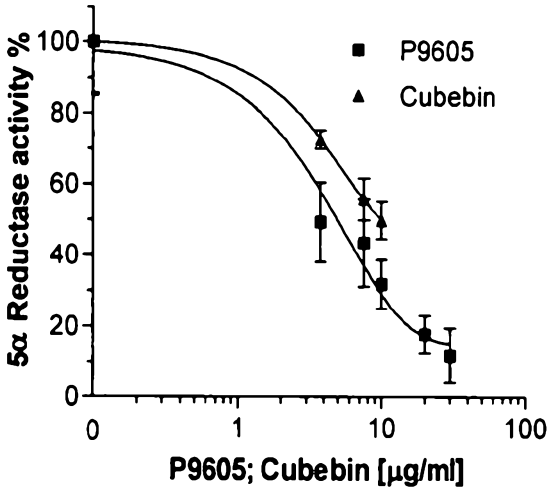

Fig. 6a

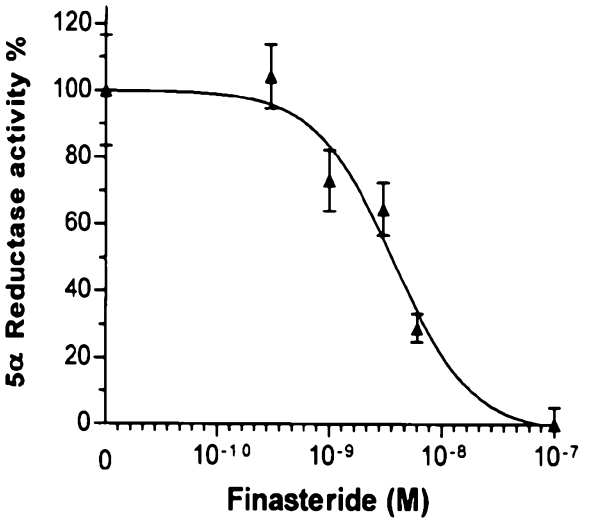

Fig. 6b

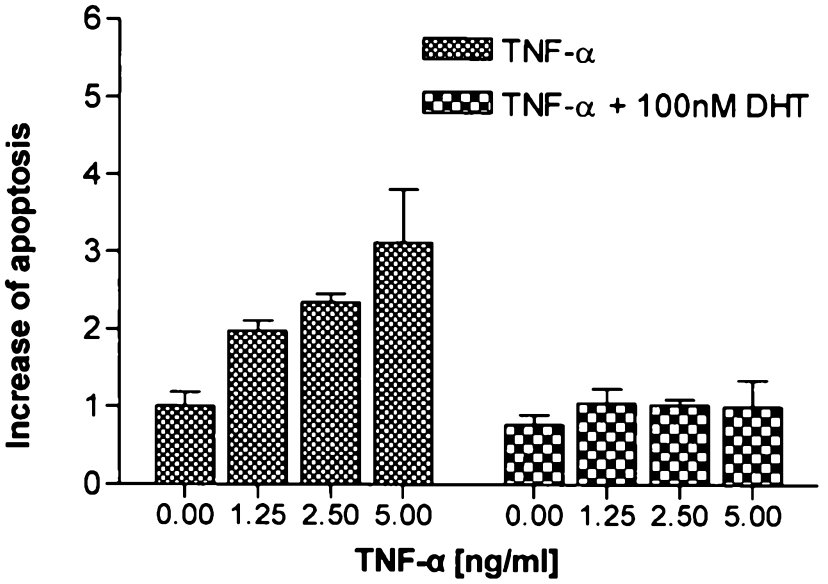

Fig. 7a

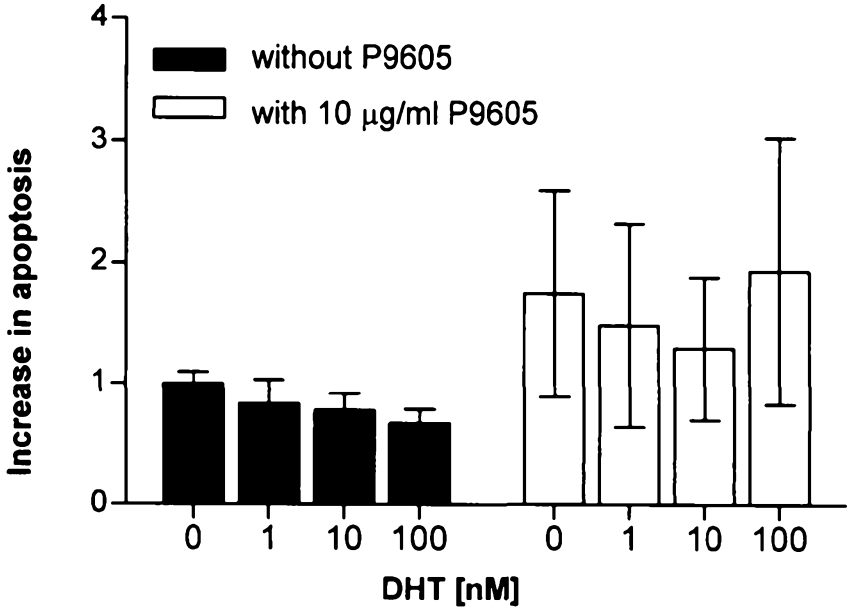
without P9605
with 10 µg/ml P9605
Increase in apoptosis
0
1
2
3
4
0
1
10
100
0
1
10
100
DHT [nM]

Fig. 7b

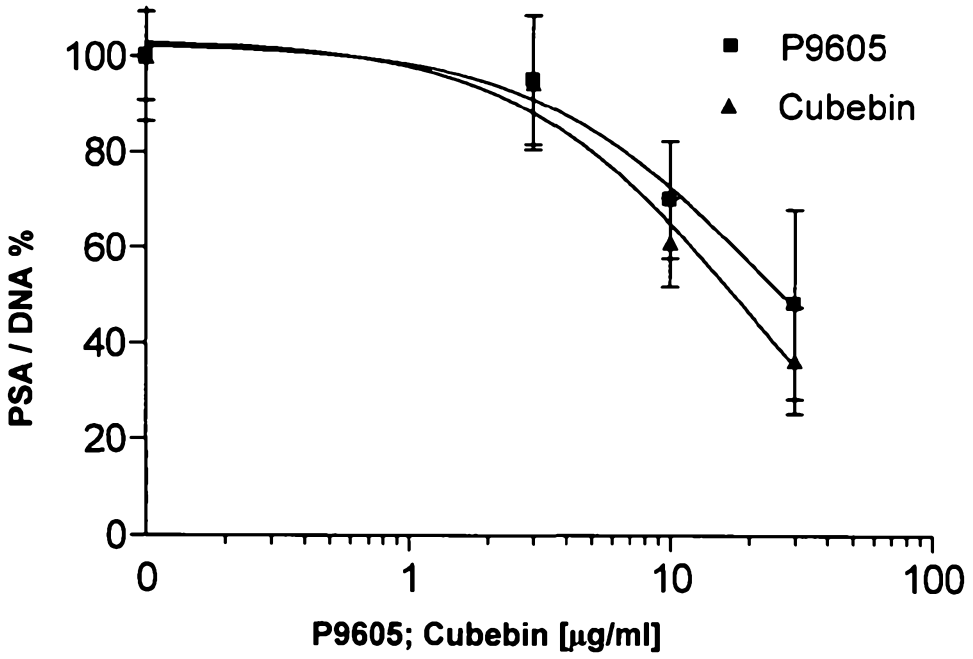
P9605
Cubebin
PSA / DNA %
0
20
40
60
80
100
0
1
10
100
P9605; Cubebin [µg/ml]

Fig. 8

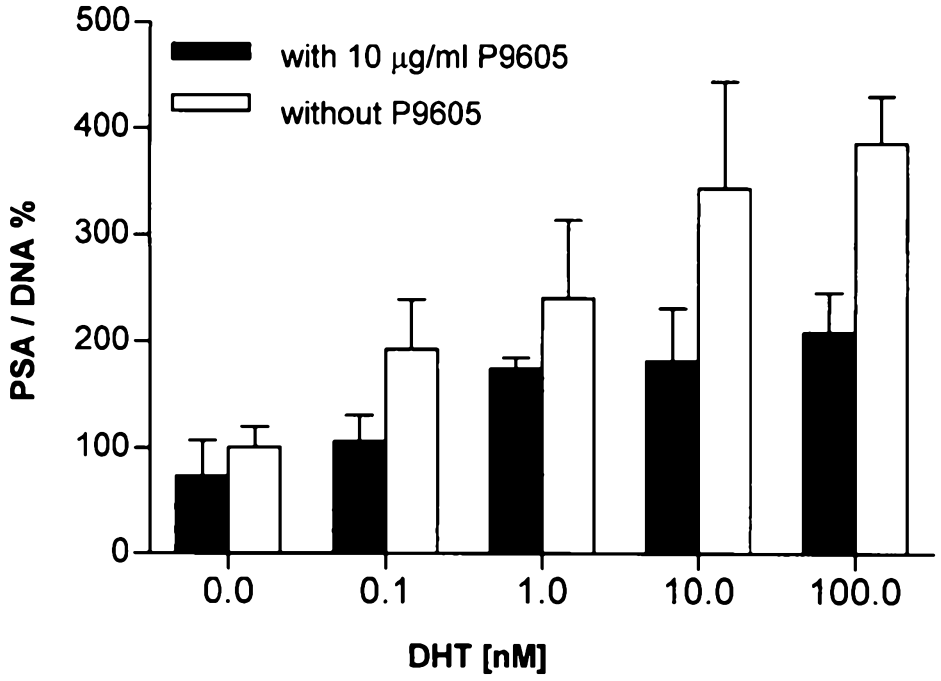
500
400
300
200
100
0
PSA / DNA %
with 10 µg/ml P9605
without P9605
0.0
0.1
1.0
10.0
100.0
DHT [nM]

Fig. 9

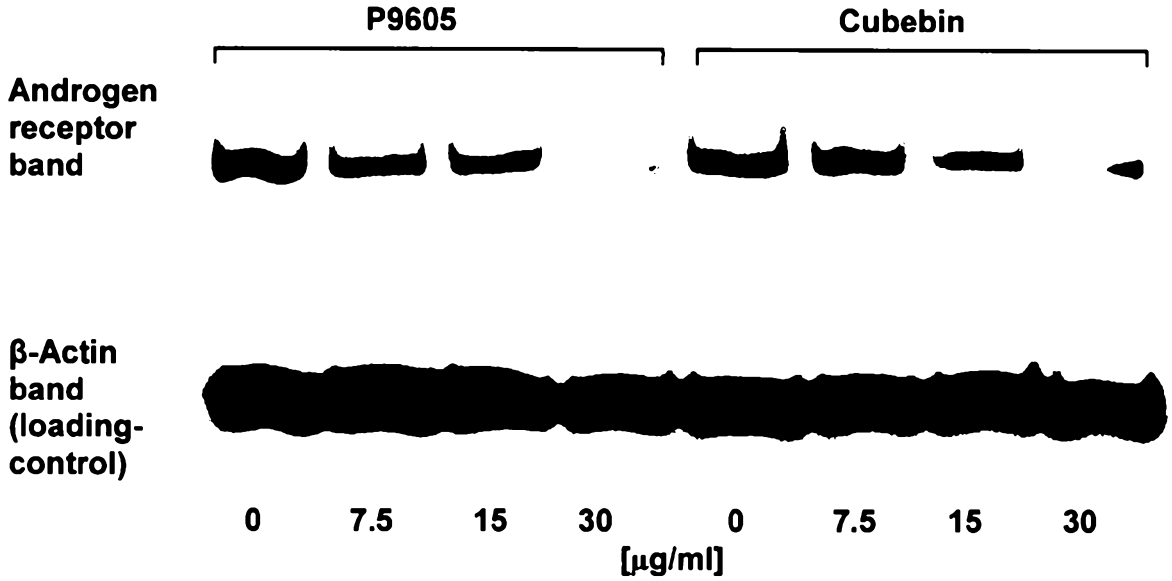
P9605
Cubebin
Androgen receptor band
β-Actin band (loading-control)
0
7.5
15
30
0
7.5
15
30
[µg/ml]

Fig. 10