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(54) **Title:** PROCESSES FOR THE PREPARATION AND ESTIMATION OF ENRICHED CALCITRIOL CONTAINING EXTRACTS FROM CESTRUM DIURNUM AND COMPOSITIONS THEREOF

(57) **Abstract:** Processes for preparation of enriched extracts containing calcitriol from the leaves and inflorescence of the Day jasmine plant i.e. Cestrum Diurnam using commonly available solvents is described. The invention further discloses quantifying the calcitriol content in the extract using simple and accurate laboratory technique such as high performance liquid chromatography (HPLC). Use of the said extract in pharmaceutical and veterinary compositions are also described.

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PROCESSES FOR THE PREPARATION AND ESTIMATION OF ENRICHED CALCITRIOL CONTAINING EXTRACTS FROM CESTRUM DIURNUM AND COMPOSITIONS THEREOF

Technical field

This invention relates to enriched extracts containing calcitriol prepared from the leaves and inflorescence of the Day jasmine plant i.e. Cestrum Diurnam. The invention particularly relates to simple processes for preparing such enriched extracts of calcitriol from Cestrum Diurnam using commonly available solvents and quantifying the calcitriol content in the extract using simple and accurate laboratory technique such as high performance liquid chromatography (HPLC). Further, the invention relates to the pharmaceutical and veterinary compositions prepared from said calcitriol enriched powder and extract for use in treating rickets, osteoporosis, psoriasis and renal osteodystrophy in humans and increasing meat weight and improving food conversion ratio(FCR) in broilers (poultry).

Background and prior art:

Calcitriol or 1 alpha, 25 – dihydroxyvitamin D3 is a hormone found in minute quantities in most living beings. Its primary function (Calcinogenic activity) is the regulation of calcium metabolism and increasing the absorption of calcium and phosphorous from intestine.

Calcitriol is used for the treatment of rickets, osteoporosis, psoriasis and renal Osteodystrophy in patients on dialysis. Calcitriol has applications in the poultry industry. It increases bird weight, improves eggshell thickness and improves feed conversion ratio (FCR). (It has application in animal husbandry)

Various plants are known to have Calcinogenic activity like Solanum malacoxylon, Trisetum flavescens, Nierembergia veitchii and Cestrum Diurnam. The Calcinogenic activity of the various extracts of these plants have been evaluated in rachitic chicks by Mello JR et al in Dtsch Tierarztl Wochenschr 1998 Jan; 105(1):25-9.

Cestrum Diurnam or Day Jasmine is a perennial plant belonging to the Solanaceae family

found widely in Hawaii island and Puerto Rico. This plant can also be cultivated in India. On commercial scale, it is being cultivated only at our own Farm (Genova Farm, Gudur Mandal, Nellore District, Andhra Pradesh, India).

Wasserman RH et al in J.Nutr 1976 Apr; 106(4):457-65 have studied the 1alpha, 25-dihydroxycholecalciferol-like activity in Cestrum Diurnam in chicks. The inclusion of dried leaf powder in a rachitogenic diet restored intestinal calcium-binding protein synthesis and increased calcium absorption in the Choleclaciferol-deficient chick.

Prema TP and Raghuramulu N have studied Free Vitamin D_3 metabolites in Cestrum diurnum leaves in Phytochemistry 37(1994) 677-681.

In Phytochemistry 1993 Sep;34 (2):511-5 Ahmad et al have published about the isolation of a new steroidal saponin named diurnoside from a methanolic extract of fresh leaves of Cestrum Diurnam. The structure of the galactopyranoside was determined by modern NMR techniques and chemical reactions.

The antimicrobial activity of the essential oil of Cestrum Diurnam was studied by Bhattacharjee et al in African Journal of Biotechnology Vol 4(4), pp 371-374, April 2005. The oil showed strong in vitro activity against P.aeruginosa and S. aureus.

Dabur Research Foundation had filed an Indian patent application claiming a synergistic herbal composition for the treatment of bone disorders comprising Calcinogenic plant parts and powder of fruits of Emblica officinalis (Amla), where the Calcinogenic plant was selected from various plants including Cestrum Diurnam and a product was introduced in the market. Subsequently the product was, however, withdrawn from the market. But till date there is no data available to indicate that calcitriol extract from Cestrum Diurnam has been formulated successfully into pharmaceutical compositions for treatment of osteoporosis and psoriasis conditions in humans and for weight increase and improved FCR in poultry/broilers.

Even though calcitriol, its formulations and uses have been documented in several patents, but till date there is no data available to indicate that calcitriol has been extracted and isolated from Cestrum Diurnam and also there is no publication of any

effort or extraction process to prepare enriched extract where therapeutically active levels have been concentrated to be analyzed to determine the exact quantity using analytical tools such as HPLC.

A usual problem encountered with extracts prepared from natural sources is that the extracts are highly coloured. Decolourisation is required to facilitate stable and safe formulation and prevent staining due to use of such formulations prepared from coloured extracts. Also, it has been seen that the process of decolourisation is so severe or harsh that it destroys the activity of the extract partially or entirely. Partial loss of activity leads to use of more amounts of the extract for the same degree of therapeutic benefit, but results in increased cost of therapy and may also increase possible side effects and toxic effects. This is a very vital point to be considered in the use of calcitriol extract from natural sources, since its application is in chronic conditions such as osteoporosis and psoriasis and in patients with renal Osteodystrophy.

Objectives of the invention:

The objective of the instant invention is to provide processes to extract calcitriol in enriched fraction from the leaves and inflorescence of Cestrum Diurnum.

Another objective of this invention is to provide processes for the extraction of enriched calcitriol by the shortest possible route using simple and easily available solvents.

Yet another objective of the invention is to provide processes of extraction including a step of decolourisation of the extract such that the extract does not lose its therapeutic properties and efficacy and also formulations of the extract do not stain skin and clothes.

It is a further objective to develop a process for preparation of enriched extract where therapeutically active levels have been concentrated to be useful in quantifying the calcitriol in the extract and to prepare pharmaceutical and veterinary formulations.

One more objective of the invention is to quantify the content of calcitriol in the extract from Cestrum Diurnam by simple, reliable, accurate and precise analytical technique

such as HPLC. This is quantified against commercially available synthetic standard. This leads to the use of accurate dosing of calcitriol in the compositions.

More objectives of the invention is to formulate pharmaceutical and veterinary compositions of the calcitriol extract in oral and external dosage forms for treatment of osteoporosis, psoriasis and renal Osteodystrophy in humans and oral formulations of the extract in dry powder form for admixture with poultry feed to increase meat weight and to improve FCR (food conversion rate) in broilers/poultry.

Summary of the invention:

The instant invention discloses processes for extraction of enriched calcitriol from the leaves of Cestrum Diurnum using ethanol and ethyl acetate as solvents. The invention further discloses a simple process to decolourise the extract obtained using activated carbon to facilitate the use of these extracts in pharmaceutical formulations without loss of therapeutic activity.

A simple, precise, accurate and reliable HPLC analytical technique to quantify the content of calcitriol in the Cestrum Diurnum extract is described.

The extract is used to prepare pharmaceutical and veterinary compositions for the treatment of various decease conditions. An external ointment/cream/gel containing the enriched extract with the base comprising petroleum jelly or any suitable ointment base is used for the treatment of psoriasis.

A soft gelatin capsule containing the enriched extract in Poly ethylene Glycol or similar medium is used for the treatment of rickets, renal osteodystrophy and osteoporosis. The extract in a dry powder form is used in the form of an admixture with poultry feed to enhance meat weight of broilers and to improve FCR.

Detailed description of the invention:

The instant invention describes processes for extraction of enriched calcitriol from the leaves of Cestrum Diurnum having a moisture content of 10-20%, using solvents

selected from ethanol and ethyl acetate. The extraction processes of the invention particularly advantageous because the processes results in calcitriol enriched extract. The invention further discloses a simple process to decolourise the extract obtained using activated carbon to facilitate the use of these extracts in pharmaceutical formulations without loss of therapeutic activity. A simple, precise, accurate and reliable HPLC analytical technique to quantify the content of calcitriol in the Cestrum Diurnum extract is described.

The present invention is described hereinafter in more details substantiating various embodiments for better understanding of the invention.

In one preferred embodiment of the invention, the leaves of Cestrum Diurnum are extracted with ethanol as first organic solvent and further treated with activated carbon for decolourisation to obtain enriched calcitriol extract. The solution is evaporated to obtain an enriched calcitriol extract. The extraction process is outlined step-wise as follows:

a) drying Cestrum Diurnam leaf to have a moisture content of 10-20%;
b) adding the first organic solvent to the leaf or leaf powder of step (a), stirring and filtering the solution;
c) repeating step (b) with residue;
d) collecting all the solutions from step (b) and (c) and concentrating and reducing the volume;
e) adding activated carbon to the above solution, mixing thoroughly and filtering the solution; and
f) evaporating the first solvent to concentrate the residual clear solution to obtain enriched calcitriol in desired concentration.

The first organic solvent used in the above process is ethanol. The calcitriol enriched extract is optionally preserved in airtight containers or in Nitrogen environment.

In another preferred embodiment of the invention, the leaves of Cestrum Diurnam are extracted using ethyl acetate as first organic solvent and further treated with activated carbon for decolourisation to obtain enriched calcitriol extract. Further, evaporating the

first organic solvent completely and dissolving the residue in ethanol as second organic solvent. The extraction process is outlined step-wise as follows:

a) drying Cestrum Diurnam leaf powder to have a moisture content of 10-20%;
b) adding first organic solvent to the leaf or leaf powder of step (a), stirring and filtering the solution;
c) repeating step (b) with residue;
d) collecting the solution of steps (b) and (c)
e) adding the activated carbon to the solution of step (d) mixing thoroughly and filtering the solution;
f) evaporating the clear solution under vacuum till the first solvent stripped to obtain the enriched extract of calcitriol at desired concentration and
g) dissolving the residue of step (f) in required amount of second organic solvent or optionally it can be dissolved in polyethylene glycol.

The first organic solvent used in the above process is ethyl acetate and the second organic solvent is ethanol. The extract of step (d) may optionally be treated with brine solution before treating with charcoal. The enriched extract is preserved in Nitrogen atmosphere/or in airtight containers.

Yet in another preferred embodiment of the invention the leaves of Cestrum Diurnam is extracted using ethanol as first organic solvent. Further, evaporating the first solvent completely under vacuum and dissolving the residue in ethyl acetate as second organic solvent to prepare second organic solution and further treated the solution with activated carbon for decolourisation to obtain clear solution. Further, the solution is concentrated by distilling the second solvent to obtain a residue. The residue is taken in required amount of first solvent to obtain enriched calcitriol extract. The extraction process is out lined step –wise as follows:

a) drying Cestrum Diurnam leaf to have a moisture content of 10-20%;
b) adding first organic solvent to the leaf or leaf powder of step (a), stirring and filtering the solution;
c) repeating step (b) with residue;

d) collecting the solution of steps (b) and (c) and distilling the first solvent under vacuum for removing the solvent completely;
e) taking the residue of step (d) into required quantity of second organic solvent and stirring well for an hour;
f) adding activated carbon to the above solution, mixing thoroughly and filtering the solution;
g) concentrating the residual solution of step (f) by evaporating second solvent completely under vacuum;
h) dissolving the residue of step (g) in required quantity of first solvent and stirring for required time.
i) evaporating first solvent under vacuum to obtain the enriched extract of calcitriol at desired concentration.

The first organic solvent used in the above process is ethanol and second solvent is ethyl acetate. The extract of step (d) may optionally be treated with brine solution before treating with charcoal. The calcitriol enriched extract is optionally stored in airtight containers or in Nitrogen Atmosphere.
Certain process parameters such as the temperature at which extraction is carried out, the period and the speed of stirring were maintained as described hereinafter. These extraction processes are further followed by an additional column purification step.

The extraction is conveniently carried out at 30-50°C by stirring for 1 to 3 hrs. The weight ratio of the dried leaf powder and the solvent used for extraction is in the range of 1:5 to 1:20. Activated carbon is used in 0.1% to 1% w/w with reference to the weight of the solution containing enriched calcitriol extract. This variation depends on the varying colour in each extraction.

Since calcitriol is sensitive to oxidation, UV and high temperatures, it is stored under nitrogen, below 40°C and away from direct sunlight.

In another preferred embodiment of the invention, the charcoal treatment of the ethanolic extract is carried out by stirring the extract using 0.1% to 1% of charcoal with reference to the amount of the extract used. The stirring is conveniently carried at room

temperature for 1 to 4 hrs filtered the solution and the solvent is distilled under reduced pressure to obtain a yellow sticky mass.

In a further embodiment of the invention, the quantification of calcitriol in the enriched extract is carried out using a simple, precise, accurate and reliable HPLC technique. The analytical method comprised of a C-18 or C-8 column and the mobile phase comprised a mixture of acetonitrile and phosphate buffer and the pH of the mobile phase is adjusted using ortho phosphoric acid to the acidic range preferably upto pH 3. The various process parameters are maintained such as flow rate, pressure, injection volume run time and the calcitriol is detected using a UV detector.

The quantity of calcitriol in one kg leaf powder is analyzed by HPLC with respect to the standard, using area method and found that ethanolic extract of one kg leaf powder comprises approximately 5 mg of calcitriol.

From 1 Kg of leaf of cestrum diurnum approximately 25mg of calcitriol can be extracted. After processing, 1 Kg dried leaf gives 150 to 200 gms of extract which contains 4-5milligrams of calcitriol. The volume of extract by using only Ethanol as solvent is 150 to 200 grams per kilo leaf and the volume of extract by using Ethanol as first solvent and Ethyl acetate as second solvent is 5 to 20 gms (process 3) Each gram of enriched extract contains approximately 25 micrograms of calcitriol. Most of the calcitriol is lost in down stream process of purification. In the process using only Ethyl acetate as solvent, each gram of extract contains up to 15 micrograms of calcitriol. This can vary between 5 and 15 depending on natural variations.

After quantifying the calcitriol in the extract, it is possible to formulate the fixed dose formulations.

Therefore, in another embodiment, the invention provides various pharmaceutical and veterinary compositions prepared using the standardized, quantified, enriched extract of calcitriol from Cestrum Diurnam.

In one aspect, the invention provides an external ointment/cream/gel containing the enriched extract with the base comprising aloe vera or petroleum jelly used for the

treatment of psoriasis. The dose range of the extract is the amount containing from 1 to 5 μg of calcitriol/ gram of the base/ml of the oil, preferably 3μg/ gram of the base/ml of the oil. The ointment/cream/gel is formulated with other ointment excipients known to a person skilled in the art selected from gelling agents/buffers/preservatives/pH adjusting agents/perfumes/fragrances/vehicles/carriers/ antioxidants, viscosity enhancers and such like.

In another aspect, the invention provides a soft gelatin capsule containing liquid fill of enriched extract in a vegetable oil or poly alkylene glycol like poly ethylene glycol is used for the treatment of rickets, renal osteodystrophy and osteoporosis. The recommended oral unit dosage of enriched extract containing calcitriol in the range of 0.1 to 0.5μg, preferably 0.25μg which is usually provided in 0.1 to 0.2 ml of the vegetable oil used in the formulations. The vegetable oil may be selected from the group consisting of corn oil, peanut oil, safflower oil, sunflower oil, soybean oil and such other suitable oils.

A softgeletin capsule is provided with calcitriol extract in polyethylene glycol as a vehicle along with capsule excipients such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT).

Further the invention provides a softgel capsule with calcitriol extract and calcium carbonate and DCP in poly ethylene glycol or vegetable oil as a vehicle along with other capsule excipients such as BHA and BHT.

Yet in another aspect, the invention provides a veterinary composition, wherein the extract in a dry powder form is used in the form of an admixture with poultry feed to enhance meat weight of broilers and to improve FCR. This has been established by conducting trials in poultry farms. The first group of broilers does not receive calcitriol and it is known that Broilers do not produce calcitriol in the first 14 to 18 days of their lives. Therefore most of the calcium and phosphorous dumped in its diet is hardly utilized. Most of the supplied elements are found to be excreted in excreta causing environmental problems. Calcitriol is essential for the absorption of Calcium and

phosphorus from intestine. Calcium is necessary for the synthesis of gut enzymes that are essential for the digestion and absorption of essential nutrients in gut.

The second group of chicks is provided with readily usable form of calcitriol which result in improvement of calcium and phosphorus absorption and utilization resulting in the production of gut enzymes that facilitate the digestion and absorption of nutrients. This kind of development normally happens from day 14 with ordinary feed. But we observed that, this development can be achieved from day 2 by providing a feed with enriched calcitriol. This gives the calcitriol supplemented chicks; a two week head start which is significant considering the life of the bird which is just 42 days. The above trials established a fact that a dose of 4mg /gm of feed results in an increase in weight by 200gms and improves FCR by 0.2, thereby concluding that the weight gain is related to meat weight and not related bone weight.

The improved FCR results in a saving of about 400 g feed/bird leading to enormous economic benefit to the poultry industry. The inclusion of enriched extract of calcitriol from Cestrum Diurnam in poultry feed also increases the poultry's resistance to infections, leading to decreased mortality, further benefiting the industry. The optimum dose of enriched calcitriol extract in dry powder form is in the dose range of 25 micrograms to 125 micrograms preferably 100 micrograms of Calcitriol. This is administered as an admixture of 4 grams of leaf powder with one kilo of regular poultry feed, which will be a dose of 100 micrograms of Calcitriol. It is also established and confirmed by our trials that a dose of 4mg of leaf powder (containing approximately 100 micrograms of calcitriol)l/gm of feed increases the weight of the bird by 200gms and improves FCR by 0.2.

The following examples, which include preferred embodiments, will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purpose of illustrative discussion of preferred embodiments of the invention.

Examples:

Example 1:

Ethanol of 95% purity was added to the dried leaf powder of Cestrum Diurnam (10:1). The solution was stirred for 1 hour at a temperature of 40°C and filtered the solution. The above step was carried out repeatedly by changing solvent and followed by filtering the solution. The combined filtered solution was distilled under vacuum to reduce the volume. Activated charcoal was added in a ratio of 0.5% w/w with respect to the weight of the solution and mixed thoroughly for 1 hour and filter the solution. The residual clear solution was concentrated by evaporation of ethanol to obtain the extract enriched with calcitriol in desired concentration.

Example 2

Ethyl acetate was added to the dried leaf powder of Cestrum Diurnam (10:1). The solution was stirred for 1 hour at a temperature of 40°C and filtered the solution. The above step was carried out repeatedly by changing solvent and followed by filtering the solution. Activated charcoal was added in a ratio of 0.1 to 0.5% w/w with respect to the weight of the solution and mixed thoroughly for 1 hour and filter the solution. The residual clear solution was concentrated by complete evaporation of ethyl acetate solvent. The residue was dissolved in Ethanol (1:5) filtered, evaporated under vacuum to obtain the extract enriched with calcitriol in desired concentration. Optionally the residue may also be dissolved in polyethylene glycol to obtain the extract enriched with calcitriol in desired concentration.

Example 3:

Ethanol of 95% purity was added to the dried leaf powder of Cestrum Diurnam (10:1). The solution was stirred for 1 hour at a temperature of 40°C and filtered the solution. The above step was carried out repeatedly by changing solvent and followed by filtering the solution. The combined solution was distilled under vacuum to remove ethanol completely. The residue was taken in ethyl acetate (1:5) and activated charcoal was added in a ratio ranging from 0.1 to 0.5% w/w with respect to the weight of the solution and mixed thoroughly for 1 hour and filter the solution. The residual clear solution was concentrated by complete evaporation of ethyl acetate to obtain a residue. The residue was dissolved in ethanol (1:5) filtered, distilled under vacuum to obtain the

enriched calcitriol extract at desired concentration. Poly ethylene Glycol can be used instead of ethanol to dissolve the extract.

Example 5

Natural Calcitriol softgel capsules (Each capsule contains 0.25mcg of calcitriol)

Ingredients	Percentage w/w
Calcitriol extract (ethanolic extract)	8.33%
Polyethylene glycol	91.65%
BHA(Butylated hydroxyl anisol)	0.01%
BHT(Butylated hydroxytoluene)	0.01%

Capsule shell composition comprises gelatin, glycerol, hydrogenated products of partially hydrolysed starch (Karoin), titanium dioxide and canthaxanthin

Example 6

Natural Calcitriol softgel capsules-calcium carbonate (Each capsule contains 0.25mcg of calcitriol and 200mg of calcium carbonate)

Ingredients	Percentage w/w
DCP + medium chain triglycerides	53.70%
Calcitriol extract (ethanolic extract)	1.85%
Calcium carbonate	44.44%
Butylated hydroxyl anisole	0.002%
Butylated hydroxyl toluene	0.002%

Calcium carbonate content may be increased to 250 or 500mg in order to make available more elemental calcium. In this context, the amounts of the ingredients given may vary accordingly. In addition, 7.5mg Zinc sulphate may also be added as additional supplement and accordingly the amounts of ingredients may vary. Capsule shell composition comprises gelatin, glycerol, hydrogenated products of partially hydrolysed starch (Karoin 83), titanium dioxide and canthaxanthin.

Dicalcium phosphate and medium chain triglycerides are mixed in sufficient quantities in medicament. Medium chain fatty triglycerides are selected from vegetable oil; for example soya bean oil.

Example 7

Natural Calcitriol ointment 30/15 Grams Tube

Ingredients	Percentage w/w
Calcitriol extract	10.0%
Petroleum jelly	75.98%
Polyethylene glycol	10.0%
Butylated hydroxyl anisol	0.01%
Butylated hydroxyl toluene	0.01%
Perfume -Lavender	2.0%
Titanium dioxide	2.0%

Process:

1. Petroleum jelly was heated upto 60degrees till it melts and was cooled by stirring the mass
2. The calcitriol extract is dissolved in required amount of polyethylene glycol, mixed well and added to the mass prepared in step 1 at 50°C with stirring.
3. Added sufficient quantities of BHA, BHT to PEG, mixed well and added to the mixture of step 2 at a temperature less than 50°C under stirring.
4. The consistency and texture of the mixture is checked and PolyEthyleneGlycol can be added if required.
5. Added titanium dioxide to a small amount of PolyEthyleneGlycol, mixed well and added to the mixture of step 4 at a temperature of less than 50°C
6. Added perfume to the above ointment mixture at a temperature of less than 45°C and allowed to cool.

Example 8:

Ingredients	parts by weight
calcitriol extract dry powder	25mcg to 125mcg
regular poultry feed	1kg

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

We claim,

1. A process for preparation of calcitriol enriched extracts from the leaves of cestrum Diurnum comprising the steps of:
    a) stirring the powder of dried leaves repeatedly using a suitable first organic solvent followed by filtering the first organic solution;
    b) concentrating the above collected solution by distilling the first solvent to reduce the volume;
    c) decolorizing the above solution using activated carbon, followed by filtering the solution and ;
    d) evaporating the first organic solvent under vacuum to obtain enriched extract of calcitriol.
2. The process as claimed in claim 1, wherein the moisture content of the dried leaves is in the range of 10 to 20%.
3. The process as claimed in claim 1, wherein said first organic solvent is selected from ethanol or ethyl acetate.
4. The process as claimed in claim 1, comprising an optional step of distilling the first organic solvent completely and adding the second organic solvent to prepare second organic solution.
5. The process as claimed in claim 1 and 4, further comprising an optional step of washing the second organic solution repeatedly with brine solution to remove the color before decolorizing the solution.
6. The process as claimed in claim 5 further comprising a step of evaporating the second organic solvent completely and adding the first organic solvent to dissolve the residue.
7. The process as claimed in claims 4 to 6, wherein said first organic solvent is ethanol.
8. The process as claimed in claims 4 to 6, wherein said second organic solvent is ethyl acetate.
9. The process as claimed in claims 1, further comprising an optional step of washing the first organic solution repeatedly with brine solution to remove the color before decolorizing the solution.

10. The process as claimed in claim 1 and 9 further comprising a step of distilling the first organic solvent completely and adding the second organic solvent to dissolve the residue.
11. The process as claimed in claims 9 and 10, wherein said first organic solvent is ethyl acetate.
12. The process as claimed in claims 9 to 10, wherein second organic solvent is ethanol.
13. The process as claimed in any of the preceding claims, wherein the weight ratio of the dried leaf powder and the solvent used for extraction is in the range of 1: 5 to 1:20.
14. The process as claimed in any of the preceding claims, wherein said activated carbon is used in 0.1% to 1% w/w with reference to the weight of the solution containing enriched calcitriol extract.
15. Calcitriol enriched extracts prepared from the leaves of Cestrum Diurnam according to claims 1 to 14.
16. Calcitriol content in the enriched extract as claimed in claim 15 is quantified using high performance liquid chromatography (HPLC).
17. A topical composition comprising from about 1 to 5μg of calcitriol obtained from the enriched extract of cestrum diurnam in a pharmaceutically acceptable base along with other suitable excipients for use in the treatment of psoriasis.
18. The topical composition as claimed in claim 17, wherein said base is selected from aloe vera or petroleum jelly or poly unsaturated oils.
19. The topical composition as claimed in claim 17 and 18 selected from the group consisting of ointment, cream or gel.
20. A soft gelatin capsule comprising from about 0.1 to 0.5μg of calcitriol obtained from the enriched extract of Cestrum Diurnam in pharmaceutically acceptable vehicle along with other suitable excipients for use in the treatment of rickets, renal osteodystrophy and osteoporosis.
21. The soft gelatin capsule as claimed in claim 20, wherein said vehicle is selected from poly alkylene glycol such as poly ethylene glycol or vegetable oil selected from corn oil, peanut oil, safflower oil, sunflower oil and soybean oil.

22. A veterinary composition comprising 4 gm dry powder of the leaves of Cestrum Diurnam in one kilo of regular poultry feed for use in poultry to increase the bird weight, to increase the resistance to infection, and to decrease mortality.
23. A method of treating a skin disorder comprising psoriasis plaques in a subject, which method includes applying a topical composition according to claims 17 to 19 to the affected area of said subject.
24. A method of treating rickets (osteomalacia) in a subject, which method comprises administering a composition according to claim 20 to the said subject.
25. A method of treating renal osteodystrophy in a subject, which method comprises administering a composition according to claim 20 to the said subject.
26. A method of treating renal osteoporosis in a subject, which method comprises administering a composition according to claim 20 to the said subject.
27. A method of treatment as claimed in claims 23 to 26, wherein said subject is a mammal.
28. A method for increasing bird weight in poultry comprising administering an effective amount of veterinary composition according to claim 22 to the said bird.
29. A method for increasing the resistance in a bird comprising administering an effective amount of veterinary composition according to claim 22 to the said bird.
30. A method to decreasing the mortality in a bird comprising administering an effective amount of veterinary composition according to claim 22 to the said bird.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/81 A61P3/14 A61K31/593

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LON-WO ESMERALDA ET AL: "Evaluation of Cestrum diurnum as an alternative of vitamin D-3 in broiler feeding" CUBAN JOURNAL OF AGRICULTURAL SCIENCE, vol. 29, no. 3, 1995, pages 349-354, XP009080332 ISSN: 0864-0408	15-30
Y	page 354; table 3	1-30

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2007	16/04/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Langer, Astrid

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WASSERMAN R H ET AL: "STUDIES ON THE 1-ALPHA 25 DI HYDROXY CHOLECALCIFEROL-LIKE ACTIVITY IN A CALCINOGENIC PLANT CESTRUM-DIURNUM IN THE CHICK" JOURNAL OF NUTRITION, vol. 106, no. 4, 1976, pages 457-465, XP009080343 ISSN: 0022-3166 cited in the application	15-30
Y	page 459, column 2 - page 460, column 2; table 1	1-30
Y	WHITEHEAD COLIN C: "A review of nutritional and metabolic factors involved in dyschondroplasia in poultry" JOURNAL OF APPLIED ANIMAL RESEARCH, vol. 13, no. 1-2, March 1998 (1998-03), pages 1-16, XP009080331 ISSN: 0971-2119 abstract; table 1	1-30
Y	FR 2 865 400 A (GREENTECH SA [FR]) 29 July 2005 (2005-07-29) page 2, line 10 - line 32; claims	1-14
Y	HAAG MARIANNE: "Vitamin D: New action mechanisms and effects" SAMJ (SOUTH AFRICAN MEDICAL JOURNAL), vol. 89, no. 11, November 1999 (1999-11), pages 1195-1199, XP009080334 ISSN: 0256-9574 page 198	17-30
X	US 5 776 461 A1 (PILLAI SREEKUMAR [US] ET AL) 7 July 1998 (1998-07-07) column 2, lines 1-12; claims	17-19
X	NEED A G ET AL: "The response to calcitriol therapy in postmenopausal osteoporotic women is a function of initial calcium absorptive status" CALCIFIED TISSUE INTERNATIONAL, vol. 61, no. 1, 1997, pages 6-9, XP002426387 ISSN: 0171-967X abstract	20,24-27
X	THOMAS M K ET AL: "Vitamin D deficiency and disorders of vitamin D metabolism" ENDOCRINOLOGY AND METABOLISM CLINICS OF NORTH AMERICA 2000 UNITED STATES, vol. 29, no. 3, 2000, pages 611-627, XP009081171 ISSN: 0889-8529 page 617 - page 620; table 2	20,21, 24-27

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2006/000397

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUDSON J Q: "IMPROVED STRATEGIES FOR THE TREATMENT OF RENAL OSTEODYSTROPHY" JOURNAL OF PHARMACY PRACTICE, TECHNOMIC PUBLISHING CO, US, vol. 15, no. 6, 2002, pages 456-471, XP009027088 ISSN: 0897-1900 page 462, column 2 - page 464, column 1; table 3	20,21, 24-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2006/000397

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-27, 29, 30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2006/000397

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2865400 A	29-07-2005	NONE	
US 5776461 A1		NONE	