



(86) Date de dépôt PCT/PCT Filing Date: 2006/08/24
(87) Date publication PCT/PCT Publication Date: 2007/09/27
(45) Date de délivrance/Issue Date: 2016/08/02
(85) Entrée phase nationale/National Entry: 2008/07/25
(86) N° demande PCT/PCT Application No.: IB 2006/002302
(87) N° publication PCT/PCT Publication No.: 2007/107811
(30) Priorité/Priority: 2006/03/22 (IN0775/DEL/2006)

(51) Cl.Int./Int.Cl. *C07C 45/80* (2006.01),
A01N 45/00 (2006.01), *A01N 65/22* (2009.01),
B01D 11/02 (2006.01), *C07C 49/513* (2006.01)

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(54) Titre : PROCEDE D'ISOLEMENT DE LA CALLITERPENONE (16ALPHA,17-DIHYDROXY-3-EXO-PHYLLOCLADANE)
(54) Title: PROCESS FOR THE ISOLATION OF CALLITERPENONE (16ALPHA, 17-DIHYDROXY-3-EXO-PHYLLOCLADANE)

(57) **Abrégé/Abstract:**

The invention provides a process for the isolation of calliterpenone (16 α , 17dihydroxy-3-oxo phyllocladane), which is a phyllocladane diterpenoid with plant growth regulating properties, from the plant *Callicarpa macrophylla*. The process of the invention isolates the calliterpenone using an organic solvent to increase yield.



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ABSTRACT

The invention provides a process for the isolation of calliterpenone (16 α , 17dihydroxy-3-oxo phyllocladane), which is a phyllocladane diterpenoid with plant growth regulating properties, from the plant *Callicarpa macrophylla*. The process of the invention isolates the calliterpenone using an organic solvent to increase yield.

PROCESS FOR THE ISOLATION OF CALLITERPENONE (16 α ,
17-DIHYDROXY-3-OXO-PHYLLOCLADANE)

Field of invention:

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The present invention relates to an improved process for the isolation of calliterpenone (16 α , 17 dihydroxy-3-oxo phyllocladane) act as a plant growth promoting phyllocladane diterpenoid.

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More particularly, it relates to an improved process for the isolation of calliterpenone (16 α , 17 dihydroxy-3-oxo phyllocladane) using specific organic solvent to increase the yield of the said compound.

Background of the invention:

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Recently phyllocladane diterpenoids isolated from plant *Callicarpa macrophylla* Vahl. (Family Verbenaceae) has been found to promote the plant growth and alleviate the effects of growth retardant allelochemicals. (Singh *et.al.* US Patent No. 6, 673, 749, 2004). These novel activities pave the utility of these compounds as natural plant growth promoters and for alleviation of the growth retarding effects produced by allelochemicals. The plant growth promoters hold commercial importance in intensive agriculture and have become most attractive in agro-business of high value crops for organic cultivation. Gibberellins (specially GA₃) is the most important group among the known plant growth regulators, auxins, cytokinins, abscisic acid and ethylene etc.

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used for enhancing the productivity of commercial crops. However, high cost restrict its application to high value crops only. Among, the phyllocladane diterpenoids from *Callicarpa macrophylla* viz. calliterpenone (16 α , 17dihydroxy-3-oxo phyllocladane) and its four derivatives (calliterpenone monoacetate, calliterpenone diacetate, isopropylidinocalliterpenone and trihydroxy calliterpenone). Most promising effects on growth promotion of roots, shoots and seed germination were demonstrated by calliterpenone (US Patent 6, 673, 749). Thus the economical isolation of calliterpenone is needed for commercial exploitation in agro-business.

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Calliterpenone and its monoacetate were first isolated by Chatterjee et al. (Tetrahedron (1972), vol. 28, pp. 4319-4323) from the dry powdered stem and leaves of *Callicarpa macrophylla*.

In that method 8 kg dried powdered leaves were extracted for 30 hrs in hexane to produce residue (1.5 gm) which on column chromatography yielded .01% and .008% calliterpenone

- 5 and its monoacetate respectively. Subramaniam et al. (Phytochemistry (1974), vol. 13, pp. 306-307) isolated calliterpenone from chloroform extract of dried leaves by column chromatography (yield not reported). Sen Gupta et al. (Journal of Indian Chemical Society (1976) (LIII), pp. 218-219) isolated calliterpenone by column chromatography of neutral fraction of rectified spirit extract (yield not reported).

- 10 In all the above reports the isolation of calliterpenone and its monoacetate have involved extraction of plant material in various solvents followed by column chromatography. Previously, from this institute Singh & Agarwal (Phytochemistry (1994) vol. 37(2), pp. 587-588 and Indian J of Chem. (1994) vol. 33b, p. 1205) isolated calliterpenone and its monoacetate by column chromatography of deposits (14 gm) obtained in water extract of
- 15 fresh leaves (50 kg) yielding 0.011% and .006% respectively. The structural studies of calliterpenone and other phyllocladane diterpenoids have been done in detail by Ahamad and Zaman (Tetrahedron Lett (1973) p. 2179), Fujita et al. (Phytochemistry (1975) vol. 14, p. 2249), Wong et al. (Acta Crystallography Section C (1991) vol. 47, p. 906), Agrawal et al. (Indian J Chem (1996) vol. 35 B, pp. 803-805) and Gui Liu et al. (Helvetica Chimica Acta
- 20 (2003) vol. 86, pp. 420-437) Yield of calliterpenone in all the reports described so far was about .01% on dry wt. basis (Chatterjee et al., (Tetrahedron (1972), vol. 28, pp. 4319-4323) or on fresh wt. basis (Singh et al., Phytochemistry (1994), vol. 13, pp. 306-307).

For economical isolation of calliterpenone extraction of plant material by various organic solvents produces dark viscous residues having total extractable constituents from which

25 isolation of calliterpenone by column chromatography produced very low yield of calliterpenone. Therefore, the efforts were made to isolate calliterpenone from the water extract of plant *Callicarpa macrophylla* using simple methods and avoiding column chromatography.

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Summary of the invention;

The invention provides a simple method for isolation of calliterpenone (16 α , 17 dihydroxy-3-oxo phyllocladane) a phyllocladane diterpenoid with the plant growth regulating properties, from plant genus *Callicarpa* wherein the recovery of calliterpenone has been increased (40
5 to 92 folds to the previous methods) and comprises boiling of water washed plant material for two to four hours in pure water or 1-5% alkaline water, concentrating the extract to half the volume under vacuum, and partitioning of the concentrate with water immiscible organic solvent at temperature ranging from 20-40°, hydrolyzing the solvent fraction with 4-10% alkali if pure water is used in first extraction step, purifying the residue by filtration through
10 celiteTM using mixture of polar & non polar solvents.

The development of this process technology will pave the way for commercial utilization of calliterpenone as a plant growth promoter. Calliterpenone produces better plant growth promoting effect than GA₃ if used in particular method and also antagonize growth retardant effects produced by allelochemicals.

15 According to one aspect of the present invention, there is provided a process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of: a. drying leaves of *Callicarpa macrophylla*; b. grinding the dry leaves of *Callicarpa macrophylla* to make a powder; c. boiling the powder with an aqueous solution for 2-3 hours to obtain an aqueous extract; wherein the aqueous solution comprises between about 1 and
20 about 5 wt % alkali; d. concentrating the aqueous extract to form a concentrated extract wherein a volume of the concentrated extract is less than a volume of the aqueous extract; e. extracting the concentrated extract with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the water immiscible organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, methylethyl
25 ether, dichloromethane and butanol; and wherein step (e) is repeated 4 or 5 4 times; f. concentrating the organic extract to form crude calliterpenone; and g. purifying the crude calliterpenone to form calliterpenone.

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According to another aspect of the present invention, there is provided a process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of: a. drying leaves of *Callicarpa macrophylla*; b. grinding dry leaves of *Callicarpa macrophylla* to make a powder; c. boiling the powder with water for 2-3 hours to obtain a water extract; d. concentrating the water extract to about half volume; e. extracting the concentrated extract 4 or 5 times with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the water immiscible organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, ethyl ether, dichloromethane and butanol; f. concentrating the organic extract and hydrolyzing the organic extract with a 4-10 % alkali methanol or ethanol solution for 2 to 3 hours to form crude calliterpenone; and g. purifying the crude calliterpenone to form calliterpenone.

According to still another aspect of the present invention, there is provided a process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of: a. drying leaves of *Callicarpa macrophylla*; b. grinding dry leaves of *Callicarpa macrophylla* to make a powder; c. boiling the powder with water for 2-3 hours to obtain a water extract; d. concentrating the water extract to about half volume; e. extracting the concentrated extract 4 or 5 times with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, ethyl ether, dichloromethane and butanol; f. concentrating the organic extract to form crude calliterpenone; and g. purifying the crude calliterpenone to form calliterpenone.

Detailed description of the invention:

Accordingly, the present invention provides an improved process for the isolation of calliterpenone (16 α , 17 dihydroxy-3-oxo phyllocladane) from plant genus *Callicarpa*, wherein the said process comprising the steps of:

a) drying the leaves of *Callicarpa macrophylla* followed by grinding to make it fine powder;

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b) heating the powder obtained from step (a) with water for 2-3 hours with constant stirring to get water extract;

c) optionally heating the powder obtained from step (a) with 1-5% alkali solution for 2-3 hours with constant stirring to get water extract;

5 d) concentrating the water extract obtained from step (b) or step (c) to half the volume;

- e) further extracting the solution obtained from step (d) with water immiscible organic solvent at temperature ranging from 20 to 40 degree C and repeating 4-5 times;
- f) pooling the extracts obtained from step (e);
- 5 g) optionally hydrolyzing the extract obtained from step (f) with 4-10% alkaline methanol or ethanol for two to three hours.
- h) drying and purifying the desired product.

10 In another embodiment of the present invention, the alkaline water is prepared by dissolving 1 to 5 % alkali selected from the group consisting of sodium or potassium hydroxide in water.

Further, in another embodiment of the present invention, the water immiscible solvents are selected from the group consisting of hydrocarbons such as pentane, hexane,
15 benzene or ethers such as diethyl ether, methyl ethyl ether or chlorinated solvents such as dichloromethane, chloroform or alcohols like butanol etc.

Yet in another embodiment of the present invention, dichloromethane is used for further extracting the water extract to increase the yield of the said calliterpenone.

20 Still in another embodiment of the present invention, the purification is carried out by absorbing the said product over celite followed by washing with 20% ethyl acetate in hexane and further with ethyl acetate and hexane in the ratio 1:1 and dried it under vacuum followed by crystallization.

25 The present invention provides a process for isolation of calliterpenone (16 α , 17 dihydroxy-3-oxo phyllocladane) a phyllocladane diterpenoid with the plant growth regulating properties from plant *Callicarpa macrophylla* Vahl. (Family Verbenaceae). Besides, growth promoting activities it also antagonizes the growth retardant effects
30 produced by allelochemicals (Singh *et.al.* US Patent No. 6, 673, 749, 2004). The economical isolation of calliterpenone is needed for commercial exploitation of this compound in agro-business. The experiments were carried out for the isolation of calliterpenone and are explained in the following examples.

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All the organic solvents viz hexane, benzene, ethyl ether, ethyl acetate, chloroform, methanol and ethanol etc. can extract calliterpenone from plant but, number of other compounds and chlorophyll is also extracted in all the extracts. Process of isolation of pure calliterpenone from these extracts are very tedious, time and labor consuming by utilizing column chromatography and to get rid of the chlorophyll present become very problematic. To avoid chlorophyll, the plant materials were extracted in water as described by Singh et al.1994, but to make the process easier and maximize the yield of calliterpenone many experiments were carried out as described in following examples.

The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of present invention.

Example -1

Plant material was collected during Jan 2004 from its natural habitat from nearby area of CIMAP Resource Center Pantnagar situated in lower Himalayan region (Uttaranchal, India). The leaves were separated from twigs and dried in shade, and a portion of the dried leaves were powdered using mixer grinder. 300 gm dried intact leaves were extracted using 10 lit capacity Clevenger's type glass apparatus with 3 lit water to remove the steam volatile part and decoction thus produced was filtered and concentrated to half of its volume and extracted repeatedly four times in 500 ml hexane, using a separating funnel. The hexane extract on concentration was found to have both calliterpenone and its monoacetate as visualized by TLC. The marc was again extracted with chloroform 4x 500 ml. The chloroform extract was also found to have similar pattern of TLC as in hexane extract. All the extracts were mixed and concentrated using rotatory film evaporator to produce 1.52 gm residue dark brown in colour. The residue showed presence of 44.8% calliterpenone in the mixture (estimation was done using HPLC (as described in example-8). On TLC (hexane: ethyl acetate 1:3 and iodine chamber) the extract showed the presence of five compounds. Two were identified as calliterpenone, calliterpenone monoacetate and 3 more compounds along with many impurities were present on the

top of the TLC plates. Pure calliterpenone was obtained by absorbing the residue over silica gel and eluting with 1l. hexane and hexane: ethyl acetate with increasing polarity, first with 10% ethyl acetate in hexane then with 20%, 30% and lastly with 50%. The last elutes on further purification by rechromatography gave pure calliterpenone. 0.34g giving 0.11 %yield. The conformity of calliterpenone was done with authentic sample obtained from our previous studies (Singh & Agarwal, Phytochemistry 37(2) 587 – 588 1994 and Indian Jour. of Chem. 33b 1205 1994).

Example -2

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To increase the amount of calliterpenone, the monoacetate present in the extract was hydrolysed as follows:

1 kg dry powdered leaf was extracted two times in 2x 5 l. water and total water extract was extracted in chloroform in liquid – liquid extractor. The extract was concentrated using rotatory film evaporator producing (11.72 gm) dark greenish black oily and sticky residue. It was taken in chloroform (150 ml) and filtered through activated charcoal. The solution obtained was successively washed with 5% sodium bicarbonate (3 x 50 ml), 5% sodium carbonate to get the neutral fraction. The CHCl₃ extract was dried under reduced pressure and taken in methanol (100 ml). Methanol extract was refluxed with 5 % NaOH in water for one hour, cooled, filtered and extracted in CHCl₃ washed several time to remove alkali. The chloroform extract was concentrated and the residue was washed with cold ether to remove yellow colour producing yellow white amorphous compound (0.89 gm) having calliterpenone as major constituents with 3 more impurities (yield .089% dry leaves wt basis). To purify the compound it was filtered through celite by absorbing over 2 g celite and making bed with 20 g celite and washing first with 500 ml. 10% ethyl acetate in hexane then with 50% ethyl acetate in hexane. 50% ethyl acetate: hexane washings on concentrating in rotatory film evaporator produced white amorphous powder 407 mg. of pure calliterpenone (yield.040% dry wt. basis). The purity of compound was 79% as estimated by HPLC as described in example-8.

Above experiment have following limitations

- In the above method chloroform has been used many times for extraction and at each extraction some emulsions are formed in which loss of compound was very much evident.
- 5 • The use of activated charcole may be avoided as the colour of the solution obtained after the treatment did not changed and of loss in compound also could not be ignored.
- During hydrolysis aqueous alkali was used, presence of water at this stage although helped in precipitation of saponified materials but during extraction in
10 CHCL₃ many impurities also got extracted.

Example - 3

To avoid these problems the experiment was repeated as below:

- 15 Fresh plant material was collected in July2004 when the plants were in their vegetative stage, and no flowering and fruiting was observed during this period. 300 gm semi dried leaves having (12% moisture)) was washed to remove all the dirt and was extracted two times in water using 10 l capacity Clevenger type glass apparatus (2x 6
20 l). . Total extract (about 12 l) was concentrated to 3 l under vacuum and was extracted in chloroform using separating funnel (5x 300 ml). All the extract were pooled and concentrated, the residue (7.02 gm) thus obtained was dissolved in methanol (100 ml) and hydrolysed with 7% methenolic NaOH (10 ml) by refluxing on water bath for three hours (hydrolysis of monoacetate was monitored by TLC). In the hydrolysed solution
25 250 ml water was added, precipitate appeared was kept for 1 h. to settle down and then filtered through Whatman No.1 filter paper. The filtrate was extracted with CHCL₃. The CHCL₃ extract was washed till neutral & concentrated. The residue obtained was washed 2 – 3 times with ethyl ether and was purified as described in previous experiment example No-3 to yield 2.4 gm calliterpenone (yield 0.92%) having 86%
30 purity (estimated as described in Example-8).

Example -4

For further simplification of the experiment and to enhance percentage of calliterpenone in the extract, the calliterpenone mono acetate present in the extract was
5 hydrolysed using 10% NaOH as follows:

50 gm dried powdered leaves were boiled in 500 ml water using reflux condenser. The water extract thus received was made alkaline by adding 10% NaOH (5 – 7 ml) heated on water bath for 30 min. with constant stirring. This solution was cooled & extracted
10 (4x100ml) in chloroform and all the extracts were mixed and concentrated and dehydrated over anhydrous sodium sulphate. The chloroform extract were concentrated to dryness, cooled and washed with solvent ether for 3 times when all the colored compounds were extracted in ether by slow washing. Almost pure calliterpenone with slight impurities at the top of the plate was observed on TLC plate, Weight of
15 compound was 0.05 g thus producing 0.1% yield. Conversion of monoacetate was complete but the yield was very low as compared to previous experiment.

Example -5

20 The experiment was further modified as follows:

Taking clue from example-3 (semi dried leaves produced better recovery of calliterpenone) dried leaves were soaked with water before processing as follows:

25 200gm dried leaves were cleaned with water and dipped for two hours before extraction in Clevenger type glass apparatus with 6 liters of water for three hours. The water extract was concentrated to 2.5lit under vacuum. The extract was further extracted four times with 250 ml in dichloromethane in a separating funnel .All the extracts were pooled together and dried under vacuum to yield, 2.3 gm residue. The dichloromethane
30 extracted water extract was further partioned in chloroform (4x 250 ml) which on concentration gave a green residue which was devoid of calliterpenone and calliterpenone monoacetate showing that all these compounds have been extracted with dichloromethane. The residue obtained from dichloromethane extract was taken in

methanol and purified as described in example - 3 to yield 0.49% calliterpenone (yield 0.49%) with 86% purity (estimated as described in Example-8).The yield of calliterpenone is less as compared to example-2. It may be due to the reason that desired compound is easily extractable from fresh plant material than the dried material.. It is important to note that calliterpenone and its acetate is not soluble in water as such but is extractable in water from the plant due to presence of certain other constituents. It is also evident that when we used dried grinded plant material it produced dark green black residue, oily in nature (example-2) and produced very less amount of calliterpenone (.04 %) on processing but, when we did not grind the leaves and dipped in water before extraction the yield increased in the present experiment (0.49%).

Example-6

To study the feasibility of the process for recovery of calliterpenone a larger batch of 15kg dried leaves (collected during September 2004) were processed as follows:

Dried leaves were extracted for two hours in water using 100 l capacity stainless steel jacketed extraction unit with heating through steam along with 70 l water. The extract was concentrated in a vacuum concentrator to about 20 l. The concentrated water extract was portioned with chloroform using liquid- liquid extractor and residue obtained in chloroform extract was hydrolysed with 10% methanolic sodium hydroxide. Solvents was removed from the hydrolysed solution using rotatory film evaporator and the residue thus obtained was washed with water untill the colour was removed. Residue was dried vacuum drier and finally purified by filtration through celite yielding 22.6 g. calliterpenone yield 0.13%.The yield was low as compared to previous examples ,this may be due to time of collection of plant material (as by Example -8).

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Example -7

Further to make the extraction more simple experiment was modified as follows:

- 5 200gm dried leaves was cleaned and dipped in water for two hours. It was extracted in Clevenger type glass apparatus along with 6 l. 1% alkaline water (60 gm sodium hydroxide dissolved in 6lit water) for three hours and the water extract thus obtained was concentrated to half its volume and extracted with dichloromethane (4x200ml). Dichloromethane extract was further processed as described in example-3 to yield
10 0.46% calliterpenone with 84% purity (estimated as described in Example-8).

The yield of calliterpenone in both the examples 5 and 7 is almost same implies that hydrolysis of calliterpenone mono acetate in the plant material or after the extraction produces similar amounts of calliterpenone but, since the processing.

- 15 In Example 6 is simple and reduces one step it is better for commercial purpose.

This process is very simple and can be utilized commercially with semidried plant material.

20 **Example-8**

- The difference recorded in yield of calliterpenone in all the above examples may be due to time of collection of plant material and the variations in the steps involved. To ascertain the content of calliterpenone present in plant, it was estimated in the leaves of
25 different collections.

25 g of dried powdered leaves of each collections January, July, and September were extracted in 200 ml 2% alkaline water (4g NaOH in 200 ml water) for two hours and the extract were portioned with dichloromethane.

- 30 The dichloromethane extract was dried, dissolved in methanol and calliterpenone was estimated using HPLC on a Shimadzu LC-10A gradient analytical HPLC equipment using mobile phase-acetonitrile: water (45:55), detection 220 nm, column Waters make Spherisorb ODS-2, 10 μ m, 250x4.6mm I.D. The calliterpenone content was found to be

0.27%, 1.03% and 0.15% in leaves collected during January, July, and September while the fruits have 0.28% but no calliterpenone was detected in the stems.

Advantages:

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The main advantages of the present invention are:

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- In present process, extraction with pure water or with alkaline water restrict the undesirable constituents. Extraction with alkaline water besides, hydrolyzing the calliterpenone monoacetate present also hydrolyze esters and neutralize the free acids present in the plant material and thus makes the process very simple producing 40 to 92 folds enhancement in yield of calliterpenone.
- Extraction & hydrolysis of monoacetate simultaneously enables the extraction of total calliterpenone present in the plant either in free form or in acetate form.
- Extraction of plant material with alkaline water reduces the processing cost as compared to extraction with solvents.
- No need of column chromatography which save time and money and make process very simple and commercially feasible.

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CLAIMS:

1. A process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of:
 - a. drying leaves of *Callicarpa macrophylla*;
 - 5 b. grinding the dry leaves of *Callicarpa macrophylla* to make a powder;
 - c. boiling the powder with an aqueous solution for 2-3 hours to obtain an aqueous extract; wherein the aqueous solution comprises between about 1 and about 5 wt % alkali;
 - d. concentrating the aqueous extract to form a concentrated extract wherein a
10 volume of the concentrated extract is less than a volume of the aqueous extract;
 - e. extracting the concentrated extract with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the water immiscible organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, methylethyl ether, dichloromethane and butanol; and wherein step (e) is repeated 4 or 5
15 times;
 - f. concentrating the organic extract to form crude calliterpenone; and
 - g. purifying the crude calliterpenone to form calliterpenone.
2. The process of claim 1, wherein the volume of the concentrated extract is about half the volume of the aqueous extract.
- 20 3. The process of claim 1 or 2, wherein step (e) further comprises combining the organic extracts.
4. The process of claim 1 or 2,

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further comprising, after step (e), combining the organic extract with an alkaline alcohol solution containing about 4 to about 10 wt % alkali, wherein the alcohol is methanol or ethanol; and

drying the organic extract.

5 5. The process of any one of claims 1 to 4, wherein the alkali is sodium hydroxide or potassium hydroxide.

6. The process of any one of claims 1 to 5, wherein the water immiscible solvent is dichloromethane.

7. The process of any one of claims 1 to 6, wherein the purifying comprises:

10 absorbing the crude calliterpenone over celiteTM;

washing the celiteTM with about 20% by volume ethyl acetate in hexane to form a first wash extract;

washing the celiteTM with 1:1 by volume ethyl acetate and hexane to form a second wash extract;

15 combining and concentrating the first and second wash extract; and

crystallizing the calliterpenone.

8. A process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of:

a. drying leaves of *Callicarpa macrophylla*;

20 b. grinding dry leaves of *Callicarpa macrophylla* to make a powder;

c. boiling the powder with water for 2-3 hours to obtain a water extract;

d. concentrating the water extract to about half volume;

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e. extracting the concentrated extract 4 or 5 times with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the water immiscible organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, ethyl ether, dichloromethane and butanol;

5 f. concentrating the organic extract and hydrolyzing the organic extract with a 4-10 % alkali methanol or ethanol solution for 2 to 3 hours to form crude calliterpenone; and

g. purifying the crude calliterpenone to form calliterpenone.

9. The process of claim 8, wherein the alkali methanol or ethanol solution comprises about 4 to 10 wt % NaOH.

10 10. A process for the isolation of calliterpenone from plant *Callicarpa macrophylla*, the process comprising the steps of:

a. drying leaves of *Callicarpa macrophylla*;

b. grinding dry leaves of *Callicarpa macrophylla* to make a powder;

c. boiling the powder with water for 2-3 hours to obtain a water extract;

15 d. concentrating the water extract to about half volume;

e. extracting the concentrated extract 4 or 5 times with a water immiscible organic solvent at a temperature ranging from 20-40°C to form an organic extract; wherein the organic solvent is selected from the group consisting of pentane, hexane, diethyl ether, ethyl ether, dichloromethane and butanol;

20 f. concentrating the organic extract to form crude calliterpenone; and

g. purifying the crude calliterpenone to form calliterpenone.

11. The process of claim 10, further comprising, after step (e), combining the organic extracts.

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12. The process of claim 10 or 11, wherein the water immiscible solvent is dichloromethane.

13. The process of any one of claims 10 to 12, wherein the purifying comprises:

absorbing the crude calliterpenone over celiteTM;

5 washing the celiteTM with about 20% by volume ethyl acetate in hexane to form a first wash extract;

washing the celiteTM with 1:1 by volume ethyl acetate and hexane to form a second wash extract;

combining and concentrating the first and second wash extract; and

10 crystallizing the calliterpenone.