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(54) Title: A METHOD FOR THE ISOLATION OF VANILLIN FROM RICE STRAW

(57) Abstract: The present invention relates to a process for the isolation of Vanillin (4-hydroxy- 3-methoxy benzaldehyde) by a convenient process (extraction with organic solvent) of pretreated cellulose from rice straw, a renewable agricultural waste. This invention relates to the extraction of vanillin using organic solvents (reusable) in a very cheap and convenient way from a renewable agriculture resource, rice straw.



A METHOD FOR THE ISOLATION OF VANILLIN FROM RICE STRAW

FIELD OF THE INVENTION

The present invention relates to a process for the isolation of Vanillin (4-hydroxy-
5 3-methoxy benzaldehyde) by modified convenient process (extraction with organic solvent) of pretreated cellulose from rice straw, a renewable agricultural waste.

This invention is also relates to the extraction of vanillin using organic solvents (reusable) in a very cheap and convenient method from a renewable agriculture resource, rice straw. The process does not involve any costly or hazardous
10 chemicals.

BACKGROUND AND PRIOR ART OF THE INVENTION

Vanillin is one of the most important and popular aromatic flavor materials used extensively in foods, beverages, perfumes, cosmetic and pharmaceuticals
15 industries. More than 10 thousand tons per year had been produced by the industries all over the world through chemical synthesis. Demands increasing extensively and existing is not sufficient for the demands. Natural vanillin is mainly obtained from *Vanilla planifolia* pods which is very much expensive compared to synthetic vanillin derived from various compounds. This 'natural' vanillin, can
20 cover only 1% of the global demand and the cost is (US \$ 4,00.00-500.00 per kilogram) [Virginia Commonwealth University News, 14.04.2003, vcunews@vcu.edu]. Alternative biotechnology-based approaches for the production are based on bioconversion of lignin, phenolic stilbenes, isoeugenol, eugenol, ferulic acid, or aromatic amino acids, and on *de novo* biosynthesis,
25 applying fungi, bacteria, plant cells, or genetically engineered microorganisms. The recent focus on vanillin production is centered on isolation of ferulic acid from agro-waste lignocellosic biomass and its subsequent conversion to vanillin. Recently, biotechnological approaches have been initiated to achieve this target. Some progress is being made in developed countries to prepare vanillin through a
30 biotechnological route from ferulate-moiety containing agricultural waste. Where, ferulic acid is first isolated from its source, which is converted to vanillin and vanillic acid using microbes [Synthesis of vanillin from a carbon source; John W. frost, U.S. Pat. No. 6,372,461].

[Formulation of vanillin from paddy straw; Noor Hasyierah, M. S.; Mohamed
35 Zulkali, M. D.; Dachyar Arbain, *JIRCAS*, 73, 160-165, 2012]

In this method ferulic acid is converted to vanillin by using *Aspergillus niger* and *Phanerochaete chrysosporium* through two steps of fermentation.

- The drawback of the method is that the extraction of ferulic acid from paddy straw was carried out via alkaline hydrolysis and was optimized through Central Composite Design (CCD) to give 4.092 g/l corresponding to 0.818% of ferulic acid. Ferulic acid was further utilized as a substrate for the biotransformation of vanillin and resulting in multiple steps.

- Yet another reference may be made to Vanillin from isoeugenol by using soil stains like *Pseudomonas aeruginosa* [Use of growing cells of *Pseudomonas aeruginosa* for synthesis of the natural vanillin via conversion of isoeugenol; Iraj Nahvi *et al*, *Iranian J.Pharm.Res.*, 10(4), 749-757, 2011]

In this method vanillin is prepared from isoeugenol by using soil stains like *Pseudomonas aeruginosa*.

- The drawback of the method is that this biotechnological route is not very economical, take long time, in multiple steps.

Yet another reference may be made to Vanillin from *Oryzae sativa* by biotransformation method using *Aspergillus niger* and *Phanerochaete* [Aromatic benzaldehyde from *oryzae sativa*; Noor Hasyierah Md. Salleh *et al*, IPCBEE, 9, 140-144, 2011]

- In this method vanillin is Vanillin from *Oryzae sativa* by biotransformation method using *Aspergillus niger* and *Phanerochaete chrysosporium* through two steps of bioengineering.

The drawback of the method is that this biotechnological route is not very economical, take more time, in multiple steps.

- Yet another reference may be made to Vanillin from agriculture waste [Process for the preparation of vanillin from agriculture waste; Naleen Borthakur; US patent No. US 7,399,889 B2, 2008]

- In this method ferulate is extracted from rice straw and that ferulate is oxidized by hydrogen peroxide in the presence of manganese acetate etc. with co-catalyst from salts of cobalt, nickel etc. for conversion of vanillin.

The drawback of the method is that it uses ferulate from rice straw, hydrogen peroxide, manganese acetate, salt of cobalt etc. to get vanillin with multi steps

reactions and vanillyl alcohol and furfural present with the vanillin. Also, the yield of the vanillin was 0.012%.

The biotechnological processes require multi-steps, heavy investment and stringent control to dictate the specific enzymes to work, and have high risk of mutation or inhibition of growth of microorganisms in presence of metabolites and are slow.

Considering the environment-polluting non-renewable petroleum crude based chemicals employed in the existing methods there is a great scope for inventing handy chemical processes that can use renewable raw materials for the synthesis of vanillin in a very convenient and cost effective method.

This invention shows a new method aimed to preparation of vanillin today must concern itself for use of renewable resources and cheap and easily available chemicals. In this invention, the ideal raw material is the supernatant obtained from the precipitation of lignin from alkali solution (1-3.5%) containing agro-wastes such as rice straw, which is cheap and abundant.

OBJECTS OF THE INVENTION

The main objective of the present invention is to provide a handy method for the isolation of vanillin from pretreated cellulose of rice straw which obviates the drawbacks of the previously reported methods known prior art as detailed above.

Another objective of the present invention is to provide a new method for the isolation of vanillin directly from the supernatant of the alkali solution after precipitation of lignin of pretreated cellulose from rice straw, a renewable agricultural waste.

Yet another objective of the present invention is to provide a new method for the isolation of vanillin by using mild reaction conditions, reagents and solvent that makes the method convenient and easiest.

Yet another objective of the present invention is to provide a process for the isolation of vanillin by directly from the supernatant of the alkali solution after precipitation of lignin of pretreated cellulose from rice straw thereby reducing the cost of the chemicals to the minimal and avoiding the release of the toxic chemicals to the environment.

SUMMARY OF THE INVENTION

Accordingly the present invention provides a method for the preparation of vanillin from rice straw which comprises

- a. mixing the rice straw with base solution under reflux conditions for a period of 3-10 hours for pretreatment, then cooling to a temperature of about 25-30° C and separating the solid mass by filtration, collecting the filtrate,
- b. acidification of the said filtrate to pH 2-3 using mineral acid to separate the lignin part from the liquid by further filtration,
- c. after filtration extracting the supernatant with an organic solvent followed by removing the moisture with anhydrous salts,
- d. separating the salts from the liquid by filtration, and drying the resultant organic layer to obtain the desired crude product,
- e. purifying, drying and further recrystallizing the crude product by using solvent to get the white crystalline solid product as vanillin,
- f. solvent used in step e is recovered and reused for further experiments,
- g. vanillin obtained from the said process has melting point 82-85 °C, with a yield in the range of 0.1 to 0.2% and has sweet flavor.

In another embodiment, rice straw is obtained by Cleaning and drying and is cut and milled, which is extracted with ethanol in a soxhlet apparatus to remove fats and coloring materials to obtain the starting material for pretreatment of rice straw.

- In yet another embodiment, base solution used for pretreatment of the rice straw is selected from the group consisting of potassium hydroxide, sodium hydroxide, calcium hydroxide and sodium carbonate and in the range of 1% in 200 ml-3.5% in 200 ml per 10 gm of rice straw used.

- In yet another embodiment, the organic solvent used is selected from the group consisting of dichloromethane, dichloroethane, methanol, ethyl acetate, particularly dichloromethane in the range of 40-80 ml for 10-20 gm of pretreated rice straw used.

In yet another embodiment, the mineral acid used is selected from the group consisting of sulphuric acid, hydrochloric acid or perchloric acid etc, particularly

sulphuric acid and in the range of 10-20 ml per 10 gm of pretreated rice straw used.

In yet another embodiment, temperature used is refluxing condition is in the range of 90-100 °C.

- 5 In yet another embodiment, anhydrous salt used is anhydrous sodium sulphate, potassium sulphate, calcium sulphate etc.

In yet another embodiment, drying is done by evaporating the solvent under reduced pressure.

- 10 In yet another embodiment, column chromatography using Hexane: ethyl acetate:: 10:1 as eluent for the purification of the product is used.

In yet another embodiment, solvent used in step e of claim 1 is selected from dichloromethane, ethanol, methanol, propanol or acetic acid.

In yet another embodiment of the present invention, the renewable agrowaste used is rice straw.

15 DETAILED DESCRIPTION OF THE INVENTION

- Rice straw a renewable resource, which on treatment under mild reaction conditions- sodium base/ potassium base /calcium hydroxide /sodium carbonate, sulphuric acid/ hydrochloric acid/perchloric acid, extracted with ethyl acetate/chloroform/ dichloromethane/ dichloroethane etc and on refluxing
20 produces vanillin in a easiest method particularly extraction with dichloromethane.

This is the new method where vanillin is produced from pretreated cellulose of rice straw a renewable resource which is convenient and cost effective. This method reported in the invention is novel and non-obvious.

- The following specific examples are given by way of illustration of the invention in
25 actual practice and therefore should not be construed to limit the scope of the present invention.

Rice straw were obtained from Mr Ramen Saikia of Bhathmora Gaon, P.O. RRL, Jorhat, P.S. Pulibor, Pin-785006, Assam India.(Please see Annexure-1)

EXAMPLE 1

- 30 Preparation of starting materials:

Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with methanol in a soxhlet apparatus to remove fats and coloring materials. This rice straw was preserved and used in subsequent experiments.

Pretreatment of Rice Straw

Rice straw (10 grams) was mixed with NaOH solution (1.5% in 200 ml) in a 500 ml round bottomed flask and refluxed for 5 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with H₂SO₄ acid (10 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with dichloromethane (40 ml) and washed the organic layer with water for 2-3 times. After that treated with anhydrous sodium sulphate to absorb the moisture content present the organic layer and filtered to separate sodium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor, melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 10 mg (0.1%).

EXAMPLE 2

Preparation of starting materials:

Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with methanol in a soxhlet apparatus to remove fats and coloring materials. This rice straw was preserved and used in subsequent experiments.

Pretreatment of Rice Straw

Rice straw (10 grams) was mixed with NaOH solution (2.0% in 200 ml) in a 500 ml round bottomed flask and refluxed for 5 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with H₂SO₄ acid (10 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was
5 filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with dichloromethane (40 ml) and washed the organic layer with water for 2-3 times. After that treated with anhydrous sodium sulphate to absorb the moisture content present the
10 organic layer and filtered to separate sodium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under
15 reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor, melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 14 mg (0.14%).

EXAMPLE 3

20 Preparation of starting materials:

Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with petroleum ether (60-80 °C) in a soxhlet apparatus to remove fats and coloring
25 materials. This rice straw was preserved and used in subsequent experiments.

Pretreatment of Rice Straw

Rice straw (10 grams) was mixed with NaOH solution (2.5% in 200 ml) in a 500 ml round bottomed flask and refluxed for 5 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with
30 H₂SO₄ acid (10 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with dichloromethane (40 ml) and washed the organic layer with water for 2-3 times. After that treated with anhydrous sodium sulphate to absorb the moisture content present the
5 organic layer and filtered to separate sodium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under
10 reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor, melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 20 mg (0.2%).

EXAMPLE 4**15 Preparation of starting materials:**

Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with petroleum ether (60-80 °C) in a soxhlet apparatus to remove fats and coloring
20 materials. This rice straw was preserved and used in subsequent experiments.

Pretreatment of Rice Straw

Rice straw (10 grams) was mixed with NaOH solution (3% in 200 ml) in a 500 ml round bottomed flask and refluxed for 5 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with
25 H₂SO₄ acid (10 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with dichloromethane (40 ml) and washed the organic layer with water for 2-3 times. After that treated
30 with anhydrous sodium sulphate to absorb the moisture content present the

organic layer and filtered to separate sodium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor, melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 12 mg (0.12%).

10 EXAMPLE 5

Preparation of starting materials:

Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with methanol in a soxhlet apparatus to remove fats and coloring materials. This rice straw was preserved and used in subsequent experiments.

Pretreatment of Rice Straw

Conversion of pretreated Rice straw to vanillin we were using the standard optimum condition 4 got from the experiments 1-7 for variation of alkalies. Rice straw (10 grams) was mixed with KOH solution (2.5% in 200 ml) in a 500 ml round bottomed flask and refluxed for 7 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with HCl acid (5 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with chloroform (40 ml) and washed the organic layer with water for 2-3 times. After that treated with anhydrous potassium sulphate to absorb the moisture content present the organic layer and filtered to separate potassium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in

the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor of melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 13 mg (0.13%).

EXAMPLE 6

Preparation of starting materials:

10 Dry rice straw 500 grams was extensively washed with distilled water in order to remove impurities (mainly dust). The materials were first dried in sunlight and cut into smaller pieces and milled to pass through a 1-2 mm mesh and extracted with ethanol in a soxhlet apparatus to remove fats and coloring materials. This rice straw was preserved and used in subsequent experiments.

15 Pretreatment of Rice Straw

Rice straw (10 grams) was mixed with Na_2CO_3 solution (2.5% in 200 ml) in a 500 ml round bottomed flask and refluxed for 9 hrs. in a oil bath. The reaction mixture was cooled, filtered to separate the solid products. The filtrate was acidified with H_2SO_4 acid (10 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was
20 filtered and collected.

Conversion of pretreated Rice Straw to Vanillin:

The filtrate obtained after filtration of lignin was extracted with methanol (80 ml) and washed the organic layer with water for 2-3 times. After that treated with anhydrous calcium sulphate to absorb the moisture content present the organic
25 layer and filtered to separate calcium sulphate. The organic solvent was evaporated under reduced pressure to get the crude product. The solvent used in the method is recovered and reused for further experiments. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product under
30 reduced pressure. For recrystallization of the product using methanol to give the

white crystalline solid product with sweet flavor of melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 11 mg (0.11%).

EXAMPLE 7

5 Recycle of the used solvents:

For using the recycled solvent in the conversion of pretreated rice straw to vanillin we were using the standard optimum condition 4 by following method.

Rice straw (10 grams) was mixed with NaOH solution (2.5% in 200 ml) in a 500 ml round bottomed flask and refluxed for 8 hrs. in a oil bath. The reaction mixture
10 was cooled, filtered to separate the solid products. The filtrate was acidified with sulphuric acid/hydrochloric acid/perchloric acid (5 ml) to maintain the pH 2-3 of the solution. Precipitated lignin was filtered and collected. The filtrate was extracted with dichloromethane/ethylacetate/chloroform/ dichloroethan (40-80 ml) and washed the organic layer with water for 2-3 times. The used solvent was
15 recovered by using rotating-evaporator to get the crude (solid) product as well as distilled solvent (36-78 ml) which was used for our further reaction systems. This way we were using the recycled solvents in our reaction systems. The crude product was purified by column chromatography technique using hexane : ethyl acetate :: 10:1 as eluent, and evaporated the solvent to get the desired product
20 under reduced pressure. For recrystallization of the product using methanol to give the white crystalline solid product with sweet flavor, melting point 82-85 °C (Reported 81-83 °C). The vanillin was estimated 20 mg (0.2%).

ADVANTAGES OF THE INVENTION

- 25 1. Pretreated cellulose of Agro-waste such as rice straw produces vanillin in a convenient and handy method.
2. In the present invention no further steps are required for vanillin preparation. After hydrolysis it gives the desired product directly.
3. The chemicals used are cheap and commercially available.
- 30 4. The extraction of vanillin from rice straw, described in the invention is a new method reported for the first time.
5. The solvent used for the reaction system, can be recycled, hence cost effective.

The claim:

1. A method for the preparation of vanillin from rice straw which comprises
 - a. mixing the rice straw with base solution under reflux conditions for a period of 3-10 hours at temperature in the range of 90-100°C for pretreatment, then cooling to a temperature of about 25-30°C and separating the solid mass by filtration, collecting the filtrate,
 - b. acidification of the said filtrate to pH 2-3 using mineral acid to separate the lignin part from the liquid by further filtration,
 - c. after filtration extracting the supernatant with an organic solvent followed by removing the moisture with anhydrous salts,
 - d. separating the salts from the liquid by filtration, and drying the resultant organic layer to obtain the desired crude product,
 - e. purifying, drying and further recrystallizing the crude product by using solvent to get the white crystalline solid product as vanillin,
 - f. solvent used in step e is recovered and reused for further experiments.
2. The process according to claim 1, wherein rice straw is obtained by cleaning and drying and is cut and milled, which is extracted with ethanol or methanol or by petroleum ether in a soxhlet apparatus to remove fats and coloring materials to obtain the starting material for pretreatment of rice straw.
3. The process according to claim 1, wherein the base solution used for pretreatment of the rice straw is selected from the group consisting of potassium hydroxide, sodium hydroxide, calcium hydroxide and sodium carbonate and in the range of 1% in 200 ml-3.5% in 200 ml per 10 gm of rice straw used.
4. The process according to claim 1, wherein the organic solvent used is selected from the group consisting of dichloromethane, dichloroethane, methanol, ethyl acetate, particularly dichloromethane in the range of 40-80 ml for 10-20 gm of pretreated rice straw used.

5. The process according to claim 1, wherein the mineral acid used is selected from the group consisting of sulphuric acid, hydrochloric acid or perchloric acid etc, particularly sulphuric acid and in the range of 10-20 ml per 10 gm of pretreated rice straw used.
- 5 6. The process according to claim 1, wherein vanillin obtained from the said process has melting point 82-85 °C, with a yield in the range of 0.1 to 0.2% and has sweet flavor.
7. The process according to claim 1, wherein the anhydrous salt used is anhydrous sodium sulphate, potassium sulphate, calcium sulphate etc.
- 10 8. The process according to claim 1, wherein drying is done by evaporating the solvent under reduced pressure.
9. The process according to claim 1, wherein column chromatography with Hexane: ethyl acetate :: 10:1 as eluent for the purification of the product is used.
- 15 10. The process according to claim 1, wherein solvent used in step e of claim 1 is selected from dichloromethane, ethanol, methanol, propanol or acetic acid.

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2015/050059

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C45/80 C07C45/81 C07C47/58 C11B9/00
ADD.

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)

C07C C11B

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 practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NOOR HASYIERAH, M.S. ET AL.: "Formulation of vanillin derived from paddy straw", JIRCAS WORKING REPORT, MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES, TSUKUBA, JP, vol. 73, 2012, pages 160-165, XP009187147, ISSN: 1341-710X cited in the application abstract; table 2 page 160 - page 161, line 11 -----	1-10
A	WO 2007/094013 A1 (COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH) 23 August 2007 (2007-08-23) cited in the application abstract; claims 1-3,21; examples 1,2 page 11, last paragraph ----- -/-	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

26 November 2015

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IN2015/050059

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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