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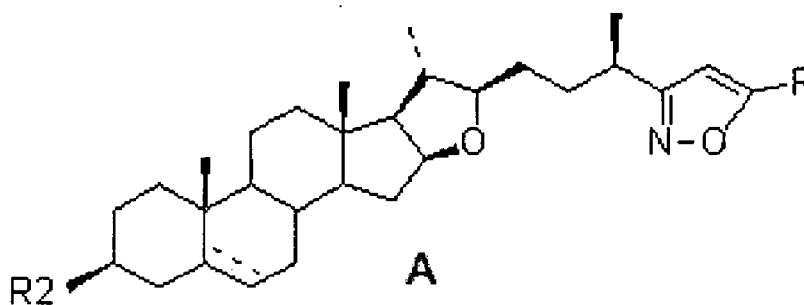
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(54) Title: DIOSGENIN ACETATE-ISOXAZOLE DERIVATIVES, PROCESS FOR PREPARATION THEREOF AND THEIR ANTIFUNGAL ACTIVITY



(57) Abstract: This invention also relates to novel class of diosgenin acetate-Isloxazole and the synthesis of diosgenin acetate-Isloxazole starting from Diosgenin and its relatives with five (5) synthetic routes, acetylation (A), F-ring opening of diosgenin acetate (B), Oxidation of the hydroxyl group on C-26 of E-ring manipulation of diosgenin acetate (C), Formation of oxime (D) and Isoxazole formation on C-26 of E-ring manipulation of diosgenin acetate (E) having potent Antifungal activity against *Alternaria alternata* fungus.

DIOSGENIN ACETATE-ISOXAZOLE DERIVATIVES, PROCESS FOR PREPARATION THEREOF AND THEIR ANTIFUNGAL ACTIVITY

FIELD OF THE INVENTION

5 This invention relates to the synthesis of a novel class of Diosgenin acetate-isoxazole starting from Diosgenin and its relatives and their antifungal activities against plant pathogenic fungus *Alternaria alternate*.

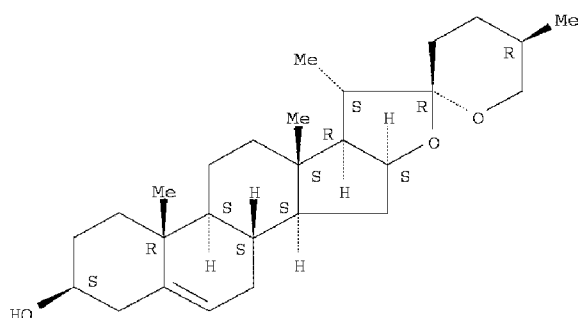
This invention also relates to the synthesis of a novel class of Diosgenin acetate-isoxazole of the formula **A** ((22 β ,25R)-3 β -acetoxyfurost-26-isoxazole) where R₂= OAc
10 from Diosgenin, viz. 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a** and **1b** where R₁= OH and with acetylene derivatives of the formula **6** where R= CH₃ or C₆H₅. The compound of formula **A** antifungal activities against plant pathogenic fungus *Alternaria alternate* prepared by the process of invention are found to have an overall yield 80% after purification. The process of the present invention does not
15 involve the use of any costly and environmentally toxic reagents and catalysts and has been developed using a non-toxic reagents under mild reaction conditions.

BACKGROUND AND PRIOR ART OF THE INVENTION

It is pertinent to note that Diosgenin acetate-isoxazole is recently gaining importance
20 owing to their potent biological activity. So far, no literature reported Diosgenin which has potent antifungal activity against plant pathogenic fungus *Alternaria alternate*.

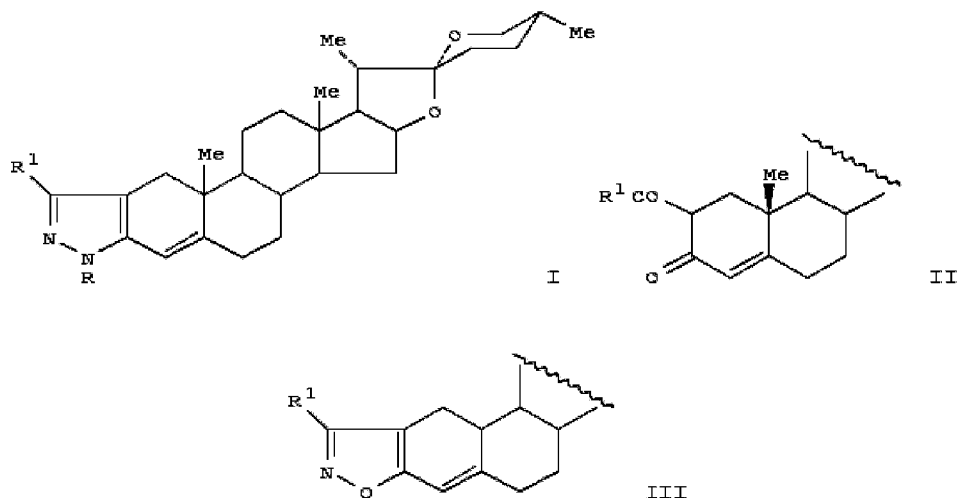
Isoxazoline derivatives of the steroid compounds solasodine and diosgenin [Prismetov, M. P.; Dzhiembaev, B. Zh.; Kiseleva, E. N.; Kharlamova, T. V., *Seriya Khimicheskaya* (2000), (3), Page No. 28-30.]

This article describes the method for the synthesis of isoxazolinolasodine and diosgenin via reaction of steroid ketones with formamide in the presence of perchloric acid with structural formula as given below:



- 5 Synthesis of pyrazoles and isoxazoles of diosgenin [M.P. Irismetov, M. I. Goryaev, and G. Yu. Tsvetkova, *Russian Journal of General Chemistry* (1976), 46(6), Page No. 1407-1409.]

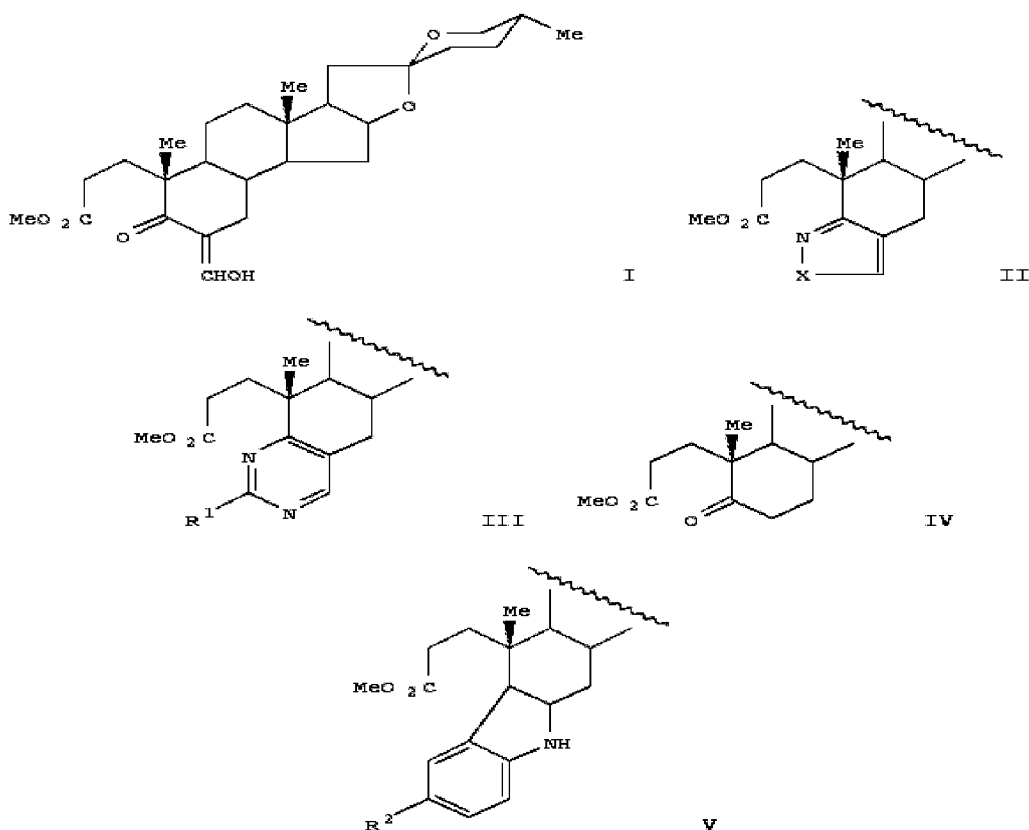
This document discloses the synthesis of pyrazoles and isoxazole derivatives of Diosgenin with the following structures.



10

Modified steroids. VIII. Synthesis and study of heterocyclic compounds made of the methyl ester of A-3,4- δ -ketonic acid from diosgenin [Irismetov, M. P.; Goryaev, M. I.; Mirzasaliev, N. A.; Rustembekova, G. B, *Izvestiya Akademii Nauk Kazakhskoi SSR, Seriya Khimicheskaya* (1982), (5), Page No. 55-59.]

This document discloses the synthesis of heterocyclic compounds made of the methyl ester of A-3,4- δ -ketonic acid from diosgenin with the following structures.



Antifungal Activity of C-27 Steroidal Saponins [Chong-Ren Yang, Ying Zhang, Melissa R. Jacob, Shabana I. Khan, Ying-Jun Zhang, and Xing-Cong Li, *Antimicrobial Agents and Chemotherapy*, (2006) 50(5), Page No. 1710-1714.]

This document discloses antifungal activity of 22 derivatives of C-27 steroidal saponins and 6 steroidal sapogenins against *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans*, and *Aspergillus fumigates* with the following structures.

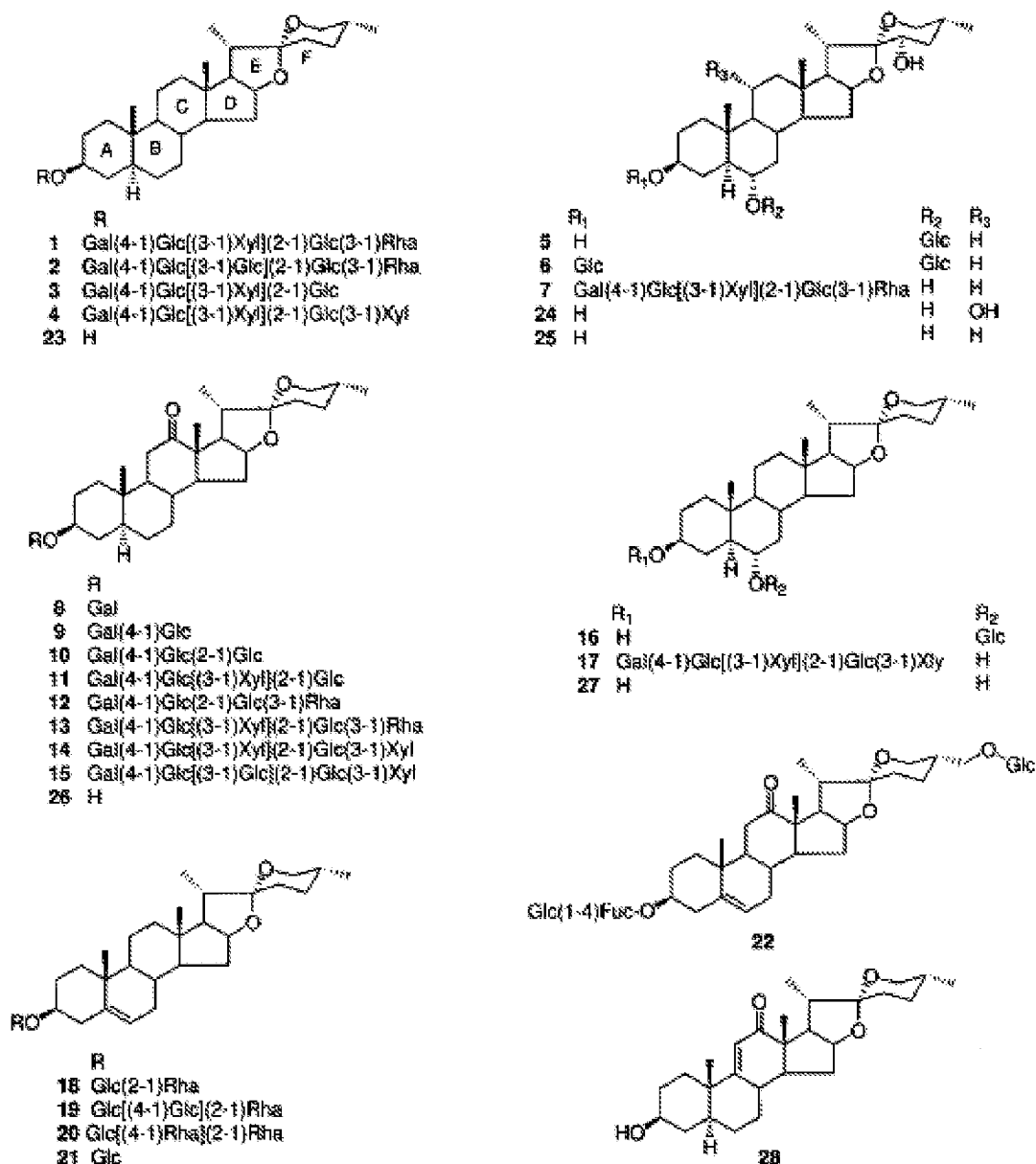


FIG. 1. Structures of compounds 1 to 28.

In vitro Antimicrobial Activity of Garhwal Himalaya Medicinal Plant *Dioscorea deltoidea* Tuber [Subhash Chandra, Sarla Saklani, Abhay P. Mishra, *International Journal of Herbal Medicine*, 2013, 1(4), Page No. 67-70.]

5 This document discloses the antimicrobial activity of the extract of *Dioscorea deltoidea*.

Thus the Diosgenin acetate-isoxazole of formula **7** as described in the present invention are completely new and novel and have so far not been reported by any other workers.

OBJECTIVES OF THE INVENTION

- 5 The main objective of the present invention, therefore, is to provide a novel process to synthesize a novel class of Diosgenin acetate-isoxazole of formula **A** starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a and 1b** where $R_1 = OH$ and with the reagent of formula **6** where $R = CH_3$ or C_6H_5 shown in the drawing (**Figure 1**).

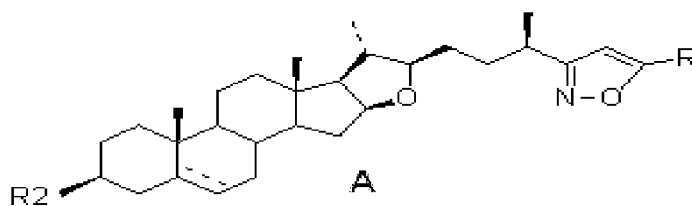
- 10 Another object of the present invention is to provide a general method of preparation of a novel class of Diosgenin acetate-isoxazole of formula **A** starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a and 1b** and their relatives where $R_1 = OH$ with five(5) reactions steps and finely with the reagent of formula **6** where $R = CH_3$ or C_6H_5 . This could be useful as potent antifungal inhibitors shown in Fig.4.

- 15 Yet another object of the present invention is to provide a novel process to synthesize a novel class of Diosgenin acetate-isoxazole of formula **A** where $R_2 = OAc$ and Ac representing acetyl group, starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a and 1b** where $R_1 = OH$ and with the reagent of formula **6** where $R = CH_3$ or C_6H_5 having potent antifungal activity against plant pathogenic fungus *Alternaria alternate*.

20

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a compound of formula **A**



wherein R₂ = OAc or OH; R = C₆H₅ or CH₃.

In an embodiment of the present invention, wherein the representative compounds comprising;

- 5 **3a.** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-ol;
- 3b.** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-ol
- 4a.** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-al;
- 4b.** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-al
- 5a.** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-oxime;
- 10 **5b.** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-oxime
- 7a.** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-methyl)isoxazole
- 7b.** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-methyl)isoxazole
- 7c.** (22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-methyl)isoxazole
- 7d.** (22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-methyl)isoxazole
- 15 **8a.** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-phenyl isoxazole)
- 8b.** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-phenyl isoxazole)
- 8c.** (22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-phenyl isoxazole)

8d. (22 β ,25R)-3 β -hydroxyfurost-5,6-dihydro-26-(5-phenyl isoxazole)

In another embodiment of the present invention, wherein diosgenin acetate -Isoxazoles exhibits good antifungal activity.

In yet another embodiment of the present invention, wherein a process for preparation of diosgenin acetate -Isoxazoles of the formula **A** wherein R₂= OAc or OH comprising the steps:

- i. optionally hydrogenating diosgenin 1 to obtain dihydrodiosgenin 1b;
- 10 ii. acetylating diosgenin 1 or dihydrodiosgenin 1b using acetic anhydride in presence of a suitable base to obtain 3 β -acetoxy dehydrodiosgenin (2a) or 3 β -acetoxy dihydrodiosgenin (2b);
- 15 iii. F-ring opening of 3 β -acetoxy dehydrodiosgenin (2a) or 3 β -acetoxy dihydrodiosgenin (2b) using sodium cyanoborohydride in acetic acid to obtain dehydro or dihydro (22 β , 25R)-3 β -acetoxyfurost-26-ol;
- iv. oxidizing dehydro or dihydro (22 β , 25R)-3 β -acetoxyfurost-26-ol using pyridinium chlorochromate (PCC) in dichloromethane to obtain C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate;
- 20 v. refluxing the mixture of C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate, hydroxyl amine hydrochloric acid, base and protic solvent at a temperature ranging between 60-95 °C for a period of 1-3 hrs, followed by cooling, washing, extracting with a water immiscible solvent and removing of solvent under reduced pressure to get
- 25 C-26-oxime-5,6 dehydro-diosgenin acetate C-26-oxime-5,6 dihydro-diosgenin acetate;

- 5
- vi. mixing the solution of C-26-oxime-5,6 dehydro-diosgenin acetate or C-26-oxime-5,6 dihydro-diosgenin acetate obtained in step (v) with phenyl acetylene or methyl acetylene, base and sodium hypochlorite in DCM (dichloromethane) at the temperature ranging between 30-50 °C under nitrogen atmosphere followed by the stirring at the temperature ranging between 30-50 °C for the period of time 25-48 hr;
- 10
- vii. adding water to the reaction mixture obtained in step (vi) then extracted with water immiscible solvent followed by washing the combined organic layer with water and brine;
- 15
- viii. drying the organic layer obtained in step (vii) followed by evaporating the solvent and purifying by column chromatography to get (22 β ,25R)-3 β -acetoxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxymurost-5,6,-dihydro-26-substituted isoxazole a compound of formula A; and
- 20
- ix. optionally hydrolyzing acetyl group of (22 β ,25R)-3 β -acetoxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxymurost-5,6,-dihydro-26-substituted isoxazole with suitable base to obtain (22 β ,25R)-3 β -hydroxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -hydroxymurost-5,6,-dihydro-26-substituted isoxazole a compounds of formula A.

In yet another embodiment of the present invention, wherein purification of the product by column chromatography with 10% ethyl acetate/hexane or 10% ethyl acetate /petroleum ether.

In yet another embodiment base is selected from the group consisting of pyridine, triethylamine.

In yet another embodiment protic solvent selected from methanol, ethanol, isoporyl alcohol.

In yet another embodiment water immiscible solvent selected from the group consisting of ethyl acetate, dichloromethane.

- 5 In yet another embodiment a composition comprising an antifungal compound of formula A or a pharmaceutically acceptable salt thereof along with pharmaceutically acceptable excipients.

In yet another embodiment compound of formula A is used for treating or preventing a fungal infection against plant pathogenic fungus *Alternaria alternate*.

- 10 In still another embodiment of the present invention, wherein a process for synthesizing or constructing novel class of Diosgenin acetate-isoxazole of formula A where R₂ = OAc or OH and Ac representing acetyl group, with the reagent of formula 6 where R = CH₃ or C₆H₅ shown in the drawing (**Figure 1**) accompanying this specification which comprises the following Steps:

- 15 **Step A** : Acetylation of diosgenin of formula 2 where R₂= OAc or OH and Ac representing the acetyl group starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula 1a and 1b and their relatives acetylated of diosgenin of formula 2 under a mild reaction conditions as per literature procedures;

- Step B** : F-ring opening of diosgenin acetate of formula 3 where R₂= OAc or OH and
20 Ac representing the acetyl group starting from 5,6 dehydro-diosgenin acetate and 5,6 dihydro-diosgenin acetate of formula 2a and 2b and their relatives under a mild reaction conditions as per literature procedures;

- Step C** : Oxidation of the hydroxyl group on C-26 of E-ring manipulation of diosgenin acetate of formula 4, starting from C-26-ol-5,6 dehydro-diosgenin acetate and C-26-ol-
25 5,6 dihydro-diosgenin acetate of formula 3 and their relatives under a mild reaction conditions as per literature procedures;

Step D : Oxime formation on C-26 of E-ring manipulation of diosgenin acetate of formula **5**, starting from C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4** and their relatives under a mild reaction conditions ie. reaction of C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4a** and **4b** with acetylene in presence of hydroxylamine hydrochloric acid, pyridine and 95% ethanol. The reaction mixture was refluxed, the progress of the reaction was monitored by TLC and the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The solvent was removed under reduced pressure to get the desired products **5**;

Step E : Isoxazole formation on C-26 of E-ring manipulation of diosgenin acetate of formula **7**, starting from C-26-oxime-5,6 dehydro-diosgenin acetate and C-26-oxime-5,6 dihydro-diosgenin acetate of formula **5a** and **5b** and of formula **6**, under a mild reaction conditions ie. reaction of 5,6 dehydro-diosgenin acetate-C-26-oxime and 5,6 dihydro-diosgenin acetate - C-26-oxime of formula **5a** and **5b** with phenyl acetylene, triethyl amine and sodium hypochlorite at 0 °C under nitrogen atmosphere and stirred 30 °C room temperature for 48 h. The progress of the reaction was monitored by TLC analysis, added water in the reaction mixture and extracted with DCM. The combined organic layer was washed with water followed by brine and dried over anhydrous Na₂SO₄. Evaporation of the solvent in high vacuum followed by column chromatography to get the pure product **7**.

In an another embodiment of the present invention, wherein process for the synthesis of a new and novel class of E-ring manipulated diosgenin acetate-Isoxazole of formula **7** starting from Diosgenin and its relatives of formula **1** can be effected by using pyridine, aniline, acetic anhydride at 70 °C for 2-7 hrs in stirring condition [**Step A**] to acetylated diosgenin of the formula **2**. F-ring opening of diosgenin acetate of formula **3** in presence of NaBH₃CN and acetic acid under argon/helium/nitrogen atmosphere in stirring condition for 50 hrs at 30 °C temperature [**Step B**]. Oxidation of the hydroxyl group on C-26 of E-ring manipulation of diosgenin acetate of formula **4** starting from C-26-ol-5,6

- dehydro-diosgenin acetate and C-26-ol-5,6 dihydro-diosgenin acetate of formula **3** and their relatives [Step C] under PCC in ice cold mixture of powdered CaCO_3 , silica and CH_2Cl_2 at 35° temperature. Formation of oxime [Step D] on C-26 of E-ring manipulation of diosgenin acetate of formula **5** starting from C-26-aldehyde-5,6
- 5 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4** and their relatives under a mild reaction conditions ie. reaction of C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4a** and **4b** with acetylene in presence of hydroxylamine hydrochloric acid, pyridine and ethanol. Isoxazoline formation on C-26 of E-ring manipulation of diosgenin acetate of
- 10 formula **A** [Step E] starting from C-26-oxime-5,6 dehydro-diosgenin acetate and C-26-oxime-5,6 dihydro-diosgenin acetate of formula **5a** and **5b** and aryl/alkyl acetylene of formula **6** under a mild reaction conditions ie. reaction of 5,6 dehydro-diosgenin acetate-C-26-oxime and 5,6 dihydro-diosgenin acetate-C-26-oxime of formula **5a** and **5b** with aryl/alkyl acetylene of formula **6**, triethyl amine and sodium hypochlorite at 0-
- 15 35°C under nitrogen/argon/helium atmosphere and stirred at 30°C temperature for 48 hrs. The progress of the reaction was monitored by TLC analysis, added water in the reaction mixture and extracted with Pet Ether/DCM/ CHCl_3 . The combined organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 and purified by column chromatography to get pure product **7**, white gummy product.
- 20 In yet another embodiment of the present invention, wherein these novel Diosgenin acetate-isoxazole of formula **A** starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a** and **1b** where $\text{R}_1 = \text{OH}$ and with the reagent of formula **6** where $\text{R} = \text{CH}_3$ or C_6H_5 have been found to show potent antifungal activity against plant pathogenic fungus *Alternaria alternate* shown in **Table 1**.
- 25 In yet another embodiment of the present invention, wherein these noval Diosgenin acetate-isoxazole of formula **A** starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a** and **1b** where $\text{R}_1 = \text{OH}$ or H and with the reagent of formula **6** where $\text{R} = \text{CH}_3$ or C_6H_5 also have been found to show with the inhibition percentage 85

as depicted in the **Fig.4** and for the starting compound 10 which was determined by the standard antifungal activity test, viz., Poison Food Technique shown in **Table 1**.

BRIEF DESCRIPTION OF THE DRAWINGS

- 5 **FIGURE 1:** *Reagents and conditions:* (a) Pyridine/ Acetic anhydride/40-90 °C/ recrystallize [MeOH/EtOH/PrOH] (b) NaBH₃CN [in CH₃COOH/ C₃H₇COOH / C₄H₉COOH (2-5 mL)]/ Inert gas atmosphere/30-60 hrs/20-30 °C/CH₂Cl₂/ Base/ purification by column chromatography (c) PCC/CaCO₃/Silica/ CH₂Cl₂/30-60 °C/ 4-12 hrs/ purification by column chromatography (d) Hydroxylamine hydrochloric acid/
- 10 Pyridine/Ethanol/ 60-100 °C/H₂O/ extract with ethyl acetate (e) DCM/Acetylene/ Triethylamine/ sodium hypochlorite/0-40 °C/ 20-48 hrs/Nitrogen atmosphere/argon atmosphere/ purification by column chromatography.

FIGURE 2: General formula according to the claim 1 is given below (Formula 1, 2, 3, 4, 5, 6 and 7):

- 15 **FIGURE 3 :** Formula of final compounds

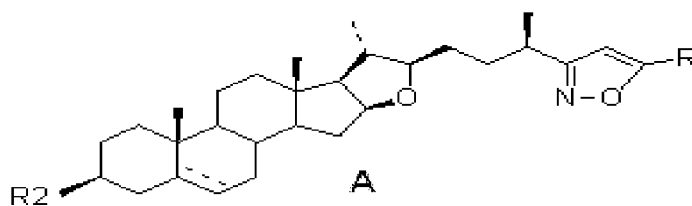
FIGURE 4: *Antifungal activity:* C= Control, E & E1= Experimental

for 7a & 7b, D= Diosgenin

Antifungal activity against plant pathogenic fungus *Alternaria alternate*.

- 20 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention is to provide a compound of formula A



wherein R₂ = OAc or OH; R = C₆H₅ or CH₃.

The representative compounds are selected from;

- 3a. (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-ol;
- 5 3b. (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-ol
- 4a. (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-al;
- 4b. (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-al
- 5a. (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-oxime;
- 5b. (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-oxime
- 10 7a. (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-methyl)isoxazole
- 7b. (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-methyl)isoxazole
- 7c. (22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-methyl)isoxazole
- 7d. (22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-methyl)isoxazole
- 8a. (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-phenyl isoxazole)
- 15 8b. (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-phenyl isoxazole)
- 8c. (22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-phenyl isoxazole)
- 8d. (22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-phenyl isoxazole)

Further a process for synthesizing or constructing a new class of Diosgenin acetate-isoxazole of formula **A** in an yield of 80% shown in the drawing (**Figure 1**) accompanying this specification which comprises the following Steps :

5 **Step A** : Acetylation of diosgenin of formula **2** where R₂= OAc or OH and Ac representing the acetyl group starting from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1** and their relatives acetylated of diosgenin of formula **2** under a mild reaction conditions as per literature procedures.

10 **Step B** : F-ring opening of diosgenin acetate of formula **3** where R₂= OAc or OH and Ac representing the acetyl group starting from 5,6 dehydro-diosgenin acetate and 5,6 dihydro-diosgenin acetate of formula **2** and their relatives under a mild reaction conditions as per literature procedures.

15 **Step C** : Oxidation of the hydroxyl group on C-26 of E-ring manipulation of diosgenin acetate of formula **4**, starting from C-26-ol-5,6 dehydro-diosgenin acetate and C-26-ol-5,6 dihydro-diosgenin acetate of formula **3** and their relatives under a mild reaction conditions as per literature procedures.

20 **Step D** : Oxime formation on C-26 of F-ring manipulation of diosgenin acetate of formula **5**, starting from C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4** and their relatives under a mild reaction conditions ie. reaction of C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde- 5,6 dihydro-diosgenin acetate of formula **4a** and **4b** with acetylene in presence of hydroxylamine hydrochloric acid, pyridine and 95% ethanol. The reaction mixture was refluxed, the progress of the reaction was monitored by TLC and the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The
25 solvent was removed under reduced pressure to get the desired products **5**.

Step E : Isoxazole formation on C-26 of E-ring manipulation of diosgenin acetate of formula **7**, starting from C-26-oxime-5,6 dehydro-diosgenin acetate and C-26-oxime-

- 5,6 dihydro-diosgenin acetate of formula **5a** and **5b** and of formula **6**, under a mild reaction conditions ie. reaction of 5,6 dehydro-diosgenin acetate-C-26-oxime and 5,6 dihydro-diosgenin acetate - C-26-oxime of formula **5a** and **5b** with phenyl acetylene, triethyl amine and sodium hypochlorite at 0 °C under nitrogen atmosphere and stirred 30 °C at room temperature for 48 hrs. The progress of the reaction was monitored by TLC analysis, added water in the reaction mixture and extracted with DCM. The combined organic layer was washed with water followed by brine and dried over anhydrous Na₂SO₄. Evaporation of the solvent in high vacuum followed by column chromatography to get pure product **7**.
- 10 This is the first ever method where these new 5,6 dehydro-diosgenin acetate -isoxazole and 5,6 dihydro-diosgenin acetate -isoxazole of formula **7a** and **7b** where R₂= OAc or OH and Ac representing the acetyl group, with the reagent of formula **6** where R= CH₃ or C₆H₅ have been found to show potent antifungal activity against plant pathogenic fungus *Alternaria alternate* with the inhibition percentage 85 and for the starting compound ie. diosgenin 10 as depicted in the **Fig.4** as determined by the standard antifungal activity test, viz., Poison Food Technique.

Antifungal activity test was performed using the plant pathogenic fungus *Alternaria alternate* with the inhibition percentage 85 as depicted in the **Fig.4** and **Table 1** as determined by the standard antifungal activity test, viz., Poison Food Technique.

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

The invention particularly relates to the synthesis of a novel class of A and E-ring manipulated Diosgenin acetate-Isoxazole of formula **A** where R₂= OAc or OH and related products shown in the diagram accompanying this specification from 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a** and **1b** where R₁= OH . In the reaction route first acetylated the diosgenin, viz. 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin [**Step A**] to acetylated diosgenin of the formula **2a** and **2b**. Then F-

ring opening of diosgenin acetate of formula **3** [Step B], Oxidation of the hydroxyl group on C-26 of E-ring manipulation of diosgenin acetate of formula **4** starting from C-26-ol-5,6 dehydro-diosgenin acetate and C-26-ol-5,6 dihydro-diosgenin acetate of formula **3** and their relatives [Step C], Formation of oxime [Step D] on C-26 of E-ring manipulation of diosgenin acetate of formula **5** starting from C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4** and their relatives under a mild reaction conditions ie. reaction of C-26-aldehyde-5,6 dehydro-diosgenin acetate and C-26-aldehyde-5,6 dihydro-diosgenin acetate of formula **4a** and **4b** and Isoxazole formation on C-26 of E-ring manipulation of diosgenin acetate of formula **7a** and **7b** [Step E] starting from C-26-oxime-5,6 dehydro-diosgenin acetate and C-26-oxime-5,6 dihydro-diosgenin acetate of formula **5a** and **5b** and acetylene derivative of formula **6** where R= CH₃ or C₆H₅.

Accordingly the present invention provides for the production of a novel class of Diosgenin acetate-Isoxazole of the formula **7a** and **7b** where R₂= OAc or OH as shown in the drawing (Figure 1) accompanying this specification from Diosgenin, viz. 5,6 dehydro-diosgenin and 5,6 dihydro-diosgenin of formula **1a** and **1b** where R₁=OH and with acetylene derivatives of the formula **6** where R= CH₃ or C₆H₅. The compound of formula **7a** and **7b** antifungal activities against plant pathogenic fungus *Alternaria alternate* as shown in Fig.4 and Table 1.

EXAMPLES

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

Example 1

Step A: Hydrogenation of Diosgenin : Diosgenin **1**, two grams in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction

mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as the eluent to furnish dihydro diosgenin compound **1b** in pure form of 95% yield.

5 **Acetylation of Diosgenin of formula 1: (synthesis of 3 β -acetoxy-diosgenin):**

Diosgenin **1**, 1.18 g and pyridine 1 ml were added to 25 ml acetic anhydride, and the mixture was heated and stirred at 90 °C for 2 hrs. The solution was poured into ice water and the precipitate was filtered. Filtrate was washed with water and dried under vacuum to yield a white solid, which was recrystallized with CH₃OH to give compound
10 **2a** with the yield of 1.06 g, (89%), mp.194 °C as colourless needle crystals.

Step B: F-ring opening of Diosgenin acetate of formula 3: ((22 β ,25R)-3 β -acetoxyfurost-26-ol : 1g NaBH₃CN was added to a 1 g solution of diosgenin acetate **2a** in acetic acid 3 ml under argon atmosphere and stirred for 60 hrs at (30 °C). After that CH₂Cl₂ was added and then Na₂CO₃ solution. The aqueous phase was extracted with
15 CH₂Cl₂ and the combined organic layer dried over anhydrous Na₂SO₄ and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3a** (white gummy liquid) with the yield of 0.716 g, 71%.

Step C: Oxidization of the formula 3 to aldehyde of formula 4:((22 β ,25R)-3 β -acetoxyfurost-26-al:
20

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO₃ 0.127 g, silica 1.0 g, and **3a**, 0.70 g in CH₂Cl₂ of 25 mL at room temperature. The reaction mixture was stirred for 12 hrs at 30 °C. Then, the reaction mixture was diluted with diethyl ether, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The
25 solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4a** with white solid of 0.492 g (70%), mp.124 °C.

Step D: Oxime derivative of Diosgenin acetate of the formula 5: ((22 β ,25R)-3 β - acetoxyfurost-26-oxime:

A mixture of **4a**, 0.2 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% ethanol 20 ml was refluxed for 1 hr, the progress of the reaction was monitored by TLC.

- 5 Then the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5a** with the yield of 0.176g (88%).

Step E: Isoxazole derivative of Diosgenin acetate of formula 7: ((22 β ,25R)-3 β - acetoxyfurost-26-(5-phenyl)isoxazole (8a**):**

- 10 To a solution of compound **5a**, 0.2 g, in DCM, 20 mL, phenyl acetylene **6**, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0 °C under nitrogen atmosphere and stirred at 30 °C for 48 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with DCM. The combined organic layer
- 15 was washed with water followed by brine and dried over anhydrous Na₂SO₄. Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) gives pure product **8a** with white gummy product of yield 0.16 g (80%).

20 **Example 2**

Step A: Hydrogenation of Diosgenin 1b:

Diosgenin **1**, two grams in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which

25 was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as the eluent to furnish dihydro diosgenin compound **1b** in pure form of 95% yield.

Acetylation of dihydrodiosgenin of formula 1b: (3 β -acetoxy- dihydrodiosgenin) :

dihydrodiosgenin **1b**, 1.18 g and pyridine 1 ml were added to 25 ml acetic anhydride, and the mixture was heated and stirred at 75 °C for 3 hrs. The solution was poured into ice water and the precipitate was filtered. Filtrate was washed with water and dried
5 under vacuum to yield a white solid, which was recrystallized with C₂H₅OH to give compound **2b** with the yield of 0.62 g, (52%), mp.194 °C as colourless needle crystals.

Step B: F-ring opening of Diosgenin acetate of formula 3 : ((22 β ,25R)-3 β -acetoxyfurost-26-ol) :

1g NaBH₃CN was added to a 1 g solution of dihydrodiosgenin acetate **2b** in propionic
10 acid 3 ml under argon atmosphere and stirred for 40 hrs at 30 °C. After that CH₂Cl₂ was added and then Na₂CO₃ solution. The aqueous phase was extracted with CH₂Cl₂ and the combined organic layer dried over anhydrous Na₂SO₄ and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3b** (white gummy liquid) with the yield of 0.491 g,
15 49%.

Step C: Oxidization of the formula 3b to aldehyde of formula 4b: ((22 β ,25R)-3 β -acetoxyfurost-26-al) :

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO₃ 0.127 g, silica 1.0 g, and **3**, 0.584 g in CH₂Cl₂ of 25 mL at 30 °C. The reaction mixture was stirred for 9 hrs
20 at temperature 40 °C. Then, the reaction mixture was diluted with diethyl ether, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4 b** with white solid of 0.26 g (44%), mp.124 °C.

Step D: Oxime derivative of dihydrodiosgenin acetate of the formula 5: ((22 β ,25R)-3 β -acetoxyfurost-26-oxime):

A mixture of **4b**, 0.196 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% ethanol 20 ml at temperature 80 °C for 1 hr, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5b** with the yield of 0.1g (51%).

Step E: Isoxazole derivative of dihydrodiosgenin acetate of formula 8b: ((22 β ,25R)-3 β -dihydroacetoxyfurost-26-(5-phenyl)isoxazole 8b):

To a solution of compound **5b**, 0.2 g, in DCM, 20 mL, phenyl acetylene **6**, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0 °C under nitrogen atmosphere and stirred at temperature 35 °C for 30 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with CHCl_3 . The combined organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **8b** with white gummy product of yield 0.1g (50%).

Example 3

Acetylation of Diosgenin of formula1: (3 β -acetoxy-diosgenin2a):

Diosgenin **1**, 1.18 g and pyridine 1 ml were added to 25 ml acetic anhydride, and the mixture was heated and stirred at 50 °C for 6 hrs. The solution was poured into ice water and the precipitate was filtered. Filtrate was washed with water and dried under vacuum to yield a white solid, which was recrystallized with $\text{C}_3\text{H}_7\text{OH}$ to give compound **2a** with the yield of 0.31 g, (26%), mp.194 °C as colourless needle crystals.

Step B: F-ring opening of Diosgenin acetate of formula 2a : ((22 β ,25R)-3 β -acetoxyfurost-26-ol) :

1g NaBH₃CN was added to a 0.31 g solution of diosgenin acetate **2a** in butanoic acid 3 ml under helium atmosphere and stirred for 30 hrs at 30 °C. After that CH₂Cl₂ was added and then Na₂CO₃ solution. The aqueous phase was extracted with ethyl acetate and the combined organic layer dried over anhydrous Na₂SO₄ and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3a** (white gummy liquid) with the yield of 0.13 g, 41%.

Step C: Oxidization of the formula 3 to aldehyde of formula 4a: ((22 β,25R)-3 β - acetoxyfurost-26-al) :

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO₃ 0.127 g, silica 1.0 g, and **3a**, 0.13 g in CH₂Cl₂ of 25 mL at 30 °C. The reaction mixture was stirred for 7 hrs at temperature 50 °C. Then, the reaction mixture was diluted with dichloromethan, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4a** with white solid of 0.05 g (38%), mp.124 °C.

Step D: Oxime derivative of Diosgenin acetate of the formula 5a: ((22 β,25R)-3 β - acetoxyfurost-26-oxime) :

A mixture of **4a**, 0.05 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% propanol 20 ml at temperature 70 °C for 2 hrs, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with chloroform. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5a** with the yield of 0.02g (40%).

Step E: Isoxazole derivative of Diosgenin acetate of formula 7 ((22 β,25R)-3 β - acetoxyfurost-26-isoxazole) :

To a solution of compound **5a**, 0.02 g, in acetone, 20 mL, methyl acetylene, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0

°C under nitrogen atmosphere and stirred at temperature 40 °C for 25 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with CHCl₃. The combined organic layer was washed with water followed by brine and dried over anhydrous Na₂SO₄.

- 5 Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **7a** with white gummy product of yield 0.009g (45%).

Example 4

Step A: Hydrogenation of Diosgenin :

- 10 Diosgenin **1**, two grams in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as the eluent to furnish dihydro diosgenin compound **1b** in pure form of 95%
15 yield.

Acetylation of dihydrodiosgenin of formula **1b**: (synthesis of 3 β -acetoxy-dihydrodiosgenin) :

- Dihydrodiosgenin **1b**, 1g and pyridine 1 ml were added to 25 ml acetic anhydride, and the mixture was heated and stirred at 90 °C for 2 hrs. The solution was poured into ice
20 water and the precipitate was filtered. Filtrate was washed with water and dried under vacuum to yield a white solid, which was recrystallized with CH₃OH to give compound **2b** with the yield of 0.83 g, (83%), mp.194 °C as colourless needle crystals.

Step B: F-ring opening of Diosgenin acetate of formula **3b**: ((22 β,25R)-3 β -acetoxyfurost-26-ol :

- 25 1g NaBH₃CN was added to a 1 g solution of dihydrodiosgenin acetate **2b** in acetic acid 3 ml under argon atmosphere and stirred for 60 hrs at (30 °C). After that CH₂Cl₂ was added and then Na₂CO₃ solution. The aqueous phase was extracted with CH₂Cl₂ and the

combined organic layer dried over anhydrous Na_2SO_4 and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3b** (white gummy liquid) with the yield of 0.70 g, 70%.

5 **Step C: Oxidization of the formula 3 to aldehyde of formula 4b: ((22 β ,25R)-3 β - acetoxyfurost-26-al:**

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO_3 0.127 g, silica 1.0 g, and **3b**, 0.70 g in CH_2Cl_2 of 25 mL at room temperature. The reaction mixture was stirred for 12 hrs at **30** °C. Then, the reaction mixture was diluted with diethyl ether,
10 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4b** with white solid of 0.42 g (60%), mp.124 °C.

15 **Step D: Oxime derivative of Diosgenin acetate of the formula 5b: ((22 β ,25R)-3 β - acetoxyfurost-26-oxime:**

A mixture of **4b**, 0.2 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% ethanol 20 ml was refluxed for 1 hr, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The solvent was removed under reduced pressure. A white gummy liquid was
20 obtained, **5b** with the yield of 0.17g (85%).

Step E: Isoxazole derivative of Diosgenin acetate of formula 7b: ((22 β ,25R)-3 β - acetoxyfurost-26-(5-methyl)isoxazole (7b):

To a solution of compound **5b**, 0.2 g, in DCM, 20 mL, methyl acetylene **6**, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0
25 °C under nitrogen atmosphere and stirred at **30** °C for 48 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with DCM. The combined organic layer

was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) gives pure product **8a** with white gummy product of yield 0.15 g (75%).

5

Example 5

Step B: F-ring opening of Diosgenin of formula **3c** : ((22 β ,25R)-3 β -hydroxyfurost-26-ol) :

1g NaBH_3CN was added to a 1 g solution of diosgenin **1** in propionic acid 3 ml under argon atmosphere and stirred for 40 hrs at 30 °C. After that CH_2Cl_2 was added and then Na_2CO_3 solution. The aqueous phase was extracted with CH_2Cl_2 and the combined organic layer dried over anhydrous Na_2SO_4 and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3c** (white gummy liquid) with the yield of 0.78 g, 78%.

15 Step C: Oxidization of the formula **3c** to aldehyde of formula **4c**: ((22 β ,25R)-3 β -hydroxyfurost-26-al) :

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO_3 0.127 g, silica 1.0 g, and **3c**, 0.8 g in CH_2Cl_2 of 25 mL at 30 °C. The reaction mixture was stirred for 7 hrs at temperature 40 °C. Then, the reaction mixture was diluted with diethyl ether, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4c** with white solid of 0.6 g (75%), mp.124 °C.

25 Step D: Oxime derivative of dehydrosdiosgenin of the formula **5c**: ((22 β ,25R)-3 β -hydroxyfurost-26-oxime):

A mixture of **4c**, 0.3 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% ethanol 20 ml at temperature 80 °C for 2 hr, the progress of the reaction was monitored

by TLC. Then the mixture was cooled with ice, washed with water and extracted with ethyl acetate. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5c** with the yield of 0.23g (76%).

Step E: Isoxazole derivative of dehydrodiosgenin of formula 8c: ((22 β ,25R)-3 β - dehydrohydroxyfurost-26-(5-phenyl)isoxazole 8c):

To a solution of compound **5c**, 0.2 g, in DCM, 20 mL, phenyl acetylene **6**, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0 °C under nitrogen atmosphere and stirred at temperature 30 °C for 20 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with CHCl_3 . The combined organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **8c** with white gummy product of yield 0.16g (80%).

15

Example 6

Step A: Hydrogenation of Diosgenin :

Diosgenin **1**, two grams in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as the eluent to furnish dihydro diosgenin compound **1b** in pure form of 95% yield.

Step B: F-ring opening of dihydrodiosgenin of formula 3d : ((22 β ,25R)-3 β - hydroxyfurost-26-ol) :

1g NaBH_3CN was added to a 1 g solution of dihydrodiosgenin **1b** in butanoic acid 3 ml under helium atmosphere and stirred for 25 hrs at 30 °C. After that CH_2Cl_2 was added

and then Na₂CO₃ solution. The aqueous phase was extracted with ethyl acetate and the combined organic layer dried over anhydrous Na₂SO₄ and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3d** (white gummy liquid) with the yield of 0.72 g, 72%.

Step C: Oxidization of the formula 3d to aldehyde of formula 4d: ((22 β,25R)-3 β - hydroxyfurost-26-al) :

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO₃ 0.127 g, silica 1.0 g, and **3b**, 0.5 g in CH₂Cl₂ of 25 mL at 30 °C. The reaction mixture was stirred for 7 hrs at temperature 50 °C. Then, the reaction mixture was diluted with dichloromethan, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4d** with white solid of 0.32 g (64%), mp.124 °C.

Step D: Oxime derivative of dihydrodiosgenin of the formula 5d: ((22 β,25R)-3 β - acetoxyfurost-26-oxime) :

A mixture of **4d**, 0.4 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% propanol 20 ml at temperature 60 °C for 2 hrs, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with chloroform. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5d** with the yield of 0.27g (67%).

Step E: Isoxazole derivative of dihydrodiosgenin of formula 8d ((22 β,25R)-3 β - hydroxyfurost-26-isoxazole) :

To a solution of compound **5d**, 0.2 g, in acetone, 20 mL, phenyl acetylene, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0 °C under nitrogen atmosphere and stirred at temperature 40 °C for 25 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml)

was added and the reaction mixture was extracted with CHCl_3 . The combined organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **8d** with white gummy product of yield 0.14g

5 (70%). **Example 7**

Step B: F-ring opening of Diosgenin of formula 3c : ((22 β ,25R)-3 β -hydroxyfurost-26-ol) :

1g NaBH_3CN was added to a 1 g solution of diosgenin **1** in propionic acid 3 ml under argon atmosphere and stirred for 30 hrs at 30 °C. After that CH_2Cl_2 was added and then
10 Na_2CO_3 solution. The aqueous phase was extracted with CH_2Cl_2 and the combined organic layer dried over anhydrous Na_2SO_4 and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3c** (white gummy liquid) with the yield of 0.73 g, 73%.

Step C: Oxidization of the formula 3c to aldehyde of formula 4c: ((22 β ,25R)-3 β -hydroxyfurost-26-al) :

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO_3 0.127 g, silica 1.0 g, and **3c**, 0.5 g in CH_2Cl_2 of 25 mL at 30 °C. The reaction mixture was stirred for 7 hrs at temperature 40 °C. Then, the reaction mixture was diluted with diethyl ether, 50.0 mL and poured through a short column containing aluminum oxide (neutral). The solvent
20 was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4c** with white solid of 0.32 g (64%), mp. 124 °C.

Step D: Oxime derivative of dehydrosdiosgenin of the formula 5c: ((22 β ,25R)-3 β -hydroxyfurost-26-oxime):

25 A mixture of **4c**, 0.3 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% ethanol 20 ml at temperature 80 °C for 2 hr, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with

ethyl acetate. The solvent was removed under reduced pressure. A white gummy liquid was obtained, **5c** with the yield of 0.20g (66%).

Step E: Isoxazole derivative of dehydrodiosgenin of formula 7c: ((22 β ,25R)-3 β - dehydrohydroxyfurost-26-(5-methyl)isoxazole 7c):

- 5 To a solution of compound **5c**, 0.2 g, in DCM, 20 mL, methyl acetylene **6**, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0 °C under nitrogen atmosphere and stirred at temperature 30 °C for 20 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with CHCl_3 . The combined organic
- 10 layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **7c** with white gummy product of yield 0.142g (71%).

15 **Example 8**

Step A: Hydrogenation of Diosgenin :

- Diosgenin **1**, two grams in 100 ml of ethanol was hydrogenated at 45 psi using 500 mg of 5% Pd/C for a period of 10 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which
- 20 was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as the eluent to furnish dihydro diosgenin compound **1b** in pure form of 95% yield.

Step B: F-ring opening of dihydrodiosgenin of formula 3d : ((22 β ,25R)-3 β - hydroxyfurost-26-ol) :

- 25 1g NaBH_3CN was added to a 1 g solution of dihydrodiosgenin **1b** in butanoic acid 3 ml under helium atmosphere and stirred for 25 hrs at 30 °C(. After that CH_2Cl_2 was added and then Na_2CO_3 solution. The aqueous phase was extracted with ethyl acetate and the

combined organic layer dried over anhydrous Na_2SO_4 and evaporated to get the product. Then the residue was purified by column chromatography (30% ethyl acetate/hexane) to give pure product **3d** (white gummy liquid) with the yield of 0.69 g, 69%.

5 **Step C: Oxidization of the formula 3d to aldehyde of formula 4d: ((22 β ,25R)-3 β - hydroxyfurost-26-al) :**

PCC, 0.273 g was added to a ice cold mixture of powdered CaCO_3 0.127 g, silica 1.0 g, and **3b**, 0.6 g in CH_2Cl_2 of 25 mL at 30 °C. The reaction mixture was stirred for 7 hrs at temperature 50 °C. Then, the reaction mixture was diluted with dichloromethan, 50.0
10 mL and poured through a short column containing aluminum oxide (neutral). The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4d** with white solid of 0.4 g (66%), mp.124 °C.

15 **Step D: Oxime derivative of dihydrodiosgenin of the formula 5d: ((22 β ,25R)-3 β - hydroxyfurost-26-oxime) :**

A mixture of **4d**, 0.4 g, hydroxylamine hydrochloric acid 0.14 g, pyridine 1 ml and 95% propanol 20 ml at temperature 60 °C for 2 hrs, the progress of the reaction was monitored by TLC. Then the mixture was cooled with ice, washed with water and extracted with chloroform. The solvent was removed under reduced pressure. A white
20 gummy liquid was obtained, **5d** with the yield of 0.24g (60%).

Step E: Isoxazole derivative of dihydrodiosgenin of formula 7d ((22 β ,25R)-3 β - hydroxyfurost-26-isoxazole) :

To a solution of compound **5d**, 0.2 g, in acetone, 20 mL, methyl acetylene, 0.072g, triethyl amine, 0.83 g and sodium hypochlorite, 10 ml (12% in water) were added at 0
25 °C under nitrogen atmosphere and stirred at temperature 40 °C for 25 hrs. The progress of the reaction was monitored by TLC analysis (15% EA/pet ether). Then, water (50 ml) was added and the reaction mixture was extracted with CHCl_3 . The combined organic

layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent in high vacuum followed by column chromatography (10% ethyl acetate/hexane) give pure product **7d** with white gummy product of yield 0.134g (67%).

5

Example 9

Biological Activity of 5,6 dehydro-diosgenin acetate-Isoxazole and 5,6-dihydro-20 diosgenin acetate -Isoxazole of formula 7 :

In Vitro Antifungal Activity Determination:

- 10 In a preferred embodiment of the present invention : The new product E-ring manipulated 5,6 dehydro - diosgenin acetate-Isoxazole and 5,6-dihydro- diosgenin acetate-Isoxazole of the formula **7a** and **7b** where $\text{R}_2 = \text{OAc}$ or OH have shown in good antifungal activity against plant pathogenic fungus *Alternaria alternate* as shown in **Fig.4** and **Table 1**.
- 15 The inhibitory effects of the sample tested in vitro on mycelia growth of *Alternaria alternata*. Poisoned Food technique using to test the antifungal activity of the product of formula **7a** and **7b** in different concentrations of 100, 200, 300, 400 and 500 ppm. Petriplates (90 mm dia.) each containing 20 ml of potato dextrose agar (PDA) medium amended with the desired concentrations of samples inoculating with test fungus. A 5
- 20 mm diameter disc of the test fungus with a corkborer, cut from the periphery of an actively growing 8 -days old culture on PDA plates and placing at the center in each treated petriplate PDA plates without product of formula **7** served as control which consist of 100, 200, 300, 400 and 500 ppm of the solvent. The experiments conducting with three replications. Then plates were kept in incubator at temp. $25 \pm 1^\circ\text{C}$. After 72
- 25 hrs. of incubation radial growth of the colony measured and calculating the mycelia growth in percentage of inhibition from mean values of colony diameter in treated and control petridishes.

Table 1 : The Inhibitory effects of the compounds on mycelial growth of *Alternaria alternata* 7days after inoculation (percent inhibition)

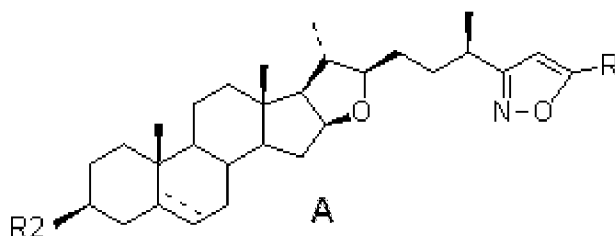
Samples	Concentrations(ppm)				
	100	200	300	400	500
(22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-methyl)isoxazole (7a)	65.2	68.0	72.2	75.0	85.0
(22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-methyl)isoxazole (7b)	64.6	67.0	70.0	78.0	85.0
(22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-methyl)isoxazole (7c)	65.1	67.9	72.0	75.1	84.8
(22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-methyl)isoxazole (7d)	65.0	67.2	71.7	75.0	84.7
(22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-phenyl)isoxazole (8a)	63.2	66.3	71.0	74.2	83.7
(22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26--(5-phenyl)isoxazole (8b)	62.5	67.0	71.2	75.0	84.0
(22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26--(5-phenyl)isoxazole (8c)	63.0	66.6	70.7	74.7	83.8
(22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26--(5-phenyl)isoxazole (8d)	64.0	66.8	70.9	74.8	83.9
Diosgenin (D)	3.3	4.0	6.5	7.0	10.0
Control (Sterile distilled water) (C)	Full growth (100%) of the fungus				

ADVANTAGES OF THE PRESENT INVENTION

1. Synthesis of a new and novel 5,6 dehydro- diosgenin acetate- Isoxazole and 5,6 dehydro- diosgenin acetate- Isoxazole.
2. Synthesis of a new and novel A and E-ring manipulated 5,6 dehydro- diosgenin acetate- Isoxazole and 5,6 dehydro- diosgenin acetate- Isoxazole.
3. Synthesized products have been found to exhibit potent antifungal activity against *Alternaria alternate*.
4. A new fungus using in the invention '*Alternaria alternate*'.
5. The chemicals used are cheap and commercially available.
6. The process is reported for the first time.

WE CLAIM:

1. A compound of formula A



5 wherein $R_2 = \text{OAc}$ or OH ; $R = \text{C}_6\text{H}_5$ or CH_3 .

2. A compound as claimed in claim 1 wherein the representative compounds are:

(22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-methyl isoxazole) (**7a**);

(22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-methyl isoxazole) (**7b**);

10 (22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-methyl isoxazole) (**7c**);

(22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-methyl isoxazole) (**7d**);

(22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-phenyl isoxazole) (**8a**);

(22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-phenyl isoxazole) (**8b**);

(22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-phenyl isoxazole) (**8c**);

15 (22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-phenyl isoxazole) (**8d**).

3. A process for a process for preparation of diosgenin acetate -Isoxazoles of the formula A where in $R_2 = \text{OAc}$ or OH ; $R = \text{C}_6\text{H}_5$ or CH_3 comprising the steps:

- i. optionally hydrogenating diosgenin 1 to obtain dihydrodiosgenin 1b;
- 5 ii. optionally acetylating diosgenin 1 or dihydrodiosgenin 1b using acetic anhydride in presence of a suitable base to obtain 3β -acetoxy dehydrodiosgenin (2a) or 3β -acetoxy dihydrodiosgenin (2b) respectively;
- 10 iii. opening F-ring of 3β -acetoxy dehydrodiosgenin (2a) or 3β -acetoxy dihydrodiosgenin (2b) using sodium cyanoborohydride in acetic acid to obtain dehydro or dihydro (22β , $25R$)- 3β -acetoxyfurost-26-ol respectively;
- 15 iv. oxidizing dehydro or dihydro (22β , $25R$)- 3β -acetoxyfurost-26-ol as obtained in step (iii) with pyridinium chlorochromate (PCC) in dichloromethane to obtain C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate respectively;
- 20 v. refluxing the mixture of C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate, hydroxyl amine hydrochloric acid, base and protic solvent at a temperature ranging between $60-95^\circ\text{C}$ for a period of 1-3 hrs, followed by cooling, washing, extracting with a water immiscible solvent and removing of solvent under reduced pressure to obtain C-26-oxime-5,6 dehydro-diosgenin acetate or C-26-oxime-5,6 dihydro-diosgenin acetate respectively;
- 25 vi. mixing the solution of C-26-oxime-5,6 dehydro-diosgenin acetate or C-26-oxime-5,6 dihydro-diosgenin acetate obtained in step (v) with phenyl acetylene or methyl acetylene, base and sodium hypochlorite

in dichloromethane at the temperature ranging between 30-50 °C under nitrogen atmosphere followed by the stirring at the temperature ranging between 30-50 °C for the period of time 25-48 hrs;

vii. adding water to the reaction mixture obtained in step(ii) followed by extraction with water immiscible solvent followed by washing the combined organic layer with water and brine;

viii. drying the organic layer obtained in step (iii) followed by evaporating the solvent and purifying by column chromatography to obtain (22 β ,25R)-3 β -acetoxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxymurost-5,6,-dihydro-26-substituted isoxazole a compound of formula A; and

ix. optionally hydrolyzing acetyl group of (22 β ,25R)-3 β -acetoxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxymurost-5,6,-dihydro-26-substituted isoxazole with suitable base to obtain (22 β ,25R)-3 β -hydroxymurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -hydroxymurost-5,6,-dihydro-26-substituted isoxazole a compounds of formula A.

4. The process as claimed in claim 3 wherein purification of the product by column chromatography with 10% ethyl acetate/hexane or 10% ethyl acetate /petroleum ether.

5. The process as claimed in claim 3, wherein the base is selected from the group consisting of pyridine, triethylamine.

6. The process as claimed in claim 3, wherein the protic solvent selected from methanol, ethanol, isopropyl alcohol.

7. The process as claimed claim 3, wherein water immiscible solvent selected from the group consisting of ethyl acetate, dichloromethane.

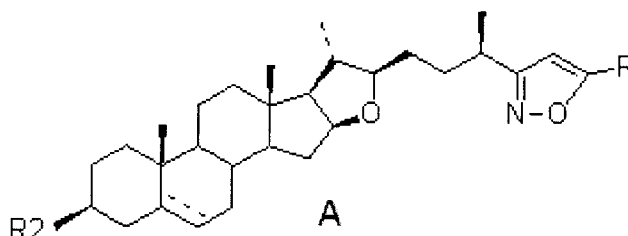
8. A compound obtained by the process as claimed in claim 3, where the compounds are
- (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-ol (3a);
- (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-ol (3b);
- 5 (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-al (4a);
- (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-al (4b);
- (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-oxime (5a);
- (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-oxime(5b).
9. A composition comprising an antifungal compound of formula A.
- 10 10. The compound of formula A as claimed in claim 1 for use in treating or preventing a fungal infection against plant pathogenic fungus *Alternaria alternate*.

AMENDED CLAIMS

received by the International Bureau on 08 July 2016 (08.07.2016)

WE CLAIM:

1. A compound of formula A



5 wherein $R_2 = \text{OAc}$ or OH ; $R = \text{C}_6\text{H}_5$ or CH_3 .

2. A compound as claimed in claim 1 wherein the representative compounds are;

(22 β ,25R)-3 β -acetoxyfurost-5,6-dehydro-26-(5-methyl isoxazole) (**7a**) ;

(22 β ,25R)-3 β -acetoxyfurost-5,6-dihydro-26-(5-methyl isooxazole) (**7b**);

10 (22 β ,25R)-3 β -hydroxyfurost-5,6-dehydro-26-(5-methyl isooxazole) (**7c**);

(22 β ,25R)-3 β -hydroxyfurost-5,6-dihydro-26-(5-methyl isooxazole) (**7d**);

(22 β ,25R)-3 β -acetoxyfurost-5,6-dehydro-26-(5-phenyl isoxazole) (**8a**)

(22 β ,25R)-3 β -acetoxyfurost-5,6-dihydro-26-(5-phenyl isooxazole) (**8b**)

(22 β ,25R)-3 β -hydroxyfurost-5,6-dehydro-26-(5-phenyl isooxazole) (**8c**);

15 (22 β ,25R)-3 β -hydroxyfurost-5,6-dihydro-26-(5-phenyl isooxazole) (**8d**)

3. A process for a process for preparation of diosgenin acetate -Isoxazoles of the formula A as claimed in claim 1 where in $R_2 = \text{OAc}$ or OH ; $R = \text{C}_6\text{H}_5$ or CH_3 comprising the steps:

- i. optionally hydrogenating diosgenin 1 to obtain dihydrodiosgenin 1b;
- 5 ii. optionally acetylating diosgenin 1 or dihydrodiosgenin 1b using acetic anhydride in presence of a suitable base to obtain 3β -acetoxy dehydrodiosgenin (2a) or 3β -acetoxy dihydrodiosgenin (2b) respectively;
- 10 iii. opening F-ring of 3β -acetoxy dehydrodiosgenin (2a) or 3β -acetoxy dihydrodiosgenin (2b) using sodium cyanoborohydride in acetic acid to obtain dehydro or dihydro (22β , $25R$)- 3β -acetoxyfurost-26-ol respectively;
- 15 iv. oxidizing dehydro or dihydro (22β , $25R$)- 3β -acetoxyfurost-26-ol as obtained in step (iii) with pyridinium chlorochromate (PCC) in dichloromethane to obtain C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate respectively;
- 20 v. refluxing the mixture of C-26-aldehyde-5,6-dehydro-diosgenin acetate or C-26-aldehyde-5,6-dihydro-diosgenin acetate, hydroxyl amine hydrochloric acid, base and protic solvent at a temperature ranging between $60-95^\circ\text{C}$ for a period of 1-3 hrs, followed by cooling, washing, extracting with a water immiscible solvent and removing of solvent under reduced pressure to obtain C-26-oxime-5,6 dehydro-diosgenin acetate or C-26-oxime-5,6 dihydro-diosgenin acetate respectively;
- 25 vi. mixing the solution of C-26-oxime-5,6 dehydro-diosgenin acetate or C-26-oxime-5,6 dihydro-diosgenin acetate obtained in step (v) with phenyl acetylene or methyl acetylene, base and sodium hypochlorite

in dichloromethane at the temperature ranging between 30-50 °C under nitrogen atmosphere followed by the stirring at the temperature ranging between 30-50 °C for the period of time 25-48 hrs;

5 vii. adding water to the reaction mixture obtained in step(ii) followed by extraction with water immiscible solvent followed by washing the combined organic layer with water and brine;

10 viii. drying the organic layer obtained in step (iii) followed by evaporating the solvent and purifying by column chromatography to obtain (22 β ,25R)-3 β -acetoxyfurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxyfurost-5,6,-dihydro-26-substituted isoxazole a compound of formula A; and

15 ix. optionally hydrolyzing acetyl group of (22 β ,25R)-3 β -acetoxyfurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -acetoxyfurost-5,6,-dihydro-26-substituted isoxazole with suitable base to obtain (22 β ,25R)-3 β -hydroxyfurost-5,6,-dehydro-26-substituted isoxazole or (22 β ,25R)-3 β -hydroxyfurost-5,6,-dihydro-26-substituted isoxazole a compounds of formula A.

4. The process as claimed in claim 3 wherein purification of the product by column chromatography with 10% ethyl acetate/hexane or 10% ethyl acetate /petroleum ether.

20 5. The process as claimed in claim 3, wherein the base is selected from the group consisting of pyridine, triethylamine.

6. The process as claimed in claim 3, wherein the protic solvent selected from methanol, ethanol, isoporyl alcohol.

25 7. The process as claimed claim 3, wherein water immiscible solvent selected from the group consisting of ethyl acetate, dichloromethane.

8. A compound obtained by the process as claimed in claim 3, where the compounds are

(22 β ,25R)-3 β -acetoxyfurost-5,6-dihydro-26-ol (3b);

(22 β ,25R)-3 β -acetoxyfurost-5,6-dihydro-26-al (4b)

(22 β ,25R)-3 β -acetoxyfurost-5,6-dihydro-26-oxime(5b)

5

9. A composition comprising an antifungal compound of formula A as claimed in claim 1.

10. The compound of formula A as claimed in claim 1 for use in treating or preventing a fungal infection against plant pathogenic fungus *Alternaria alternate*.

STATEMENT UNDER ARTICLE 19(1)

With reference to the search report and written opinion of the International Searching Authority [ISA/EP], we are suitably amending the claims to improve their clarity and to establish the novelty and inventive step in the present application.

1. Claims 3, 8 and claim 9 have been suitably amended.
2. Any addition in the claims has been indicated by underline and any deletion in the claims has been indicated by strike through.

The Applicant undertakes that no new subject matter has been added in claims and the amended claims do not go beyond the disclosure of the international application as-filed and have no impact on the description and drawings as filed.

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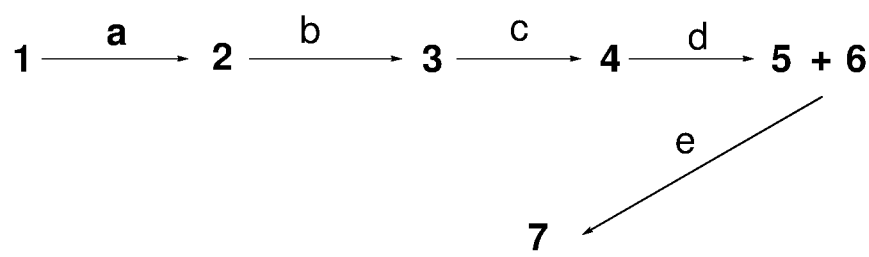


FIG. 1

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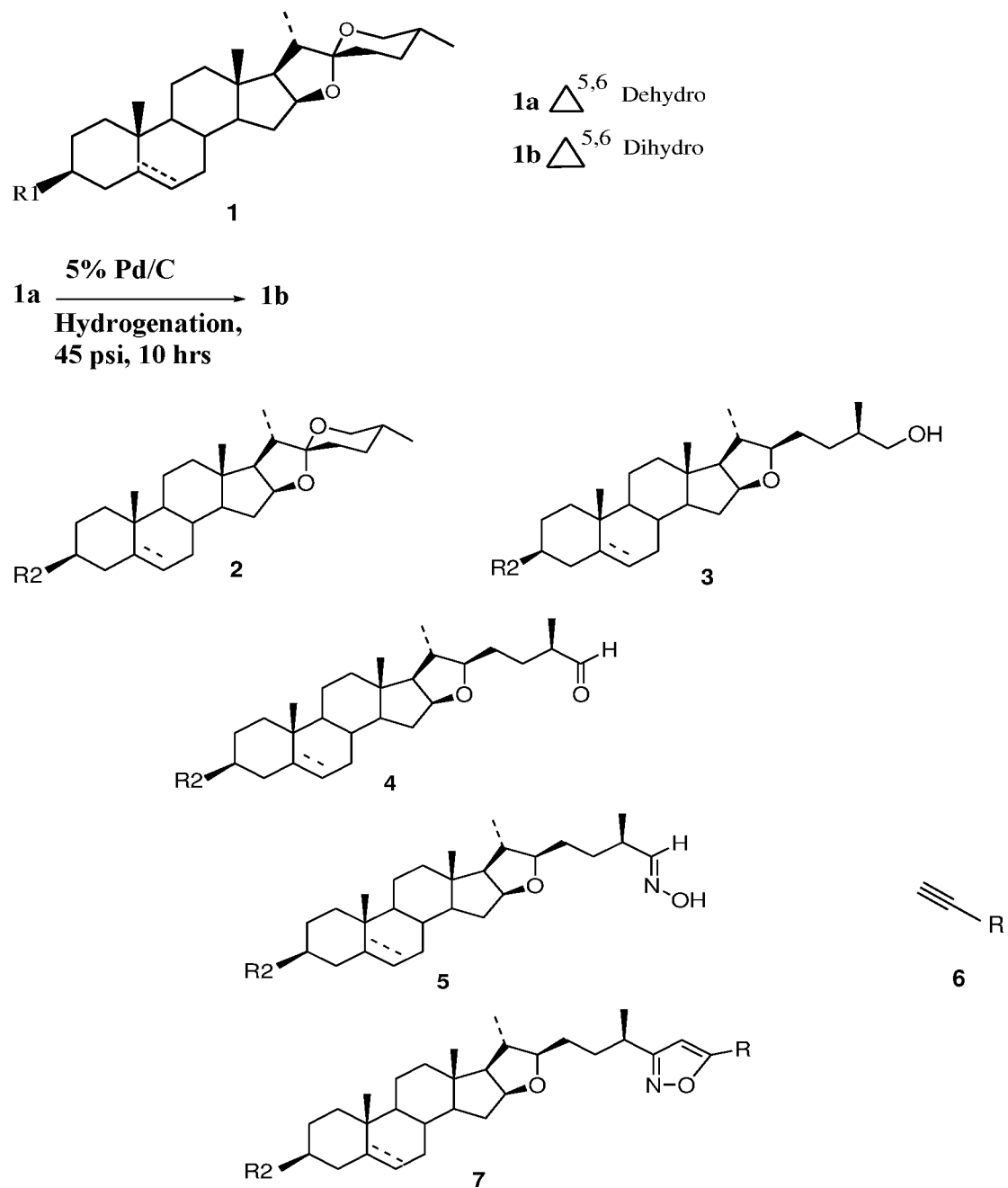
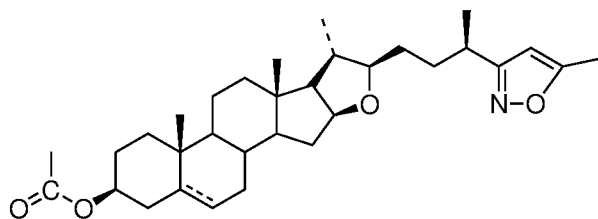
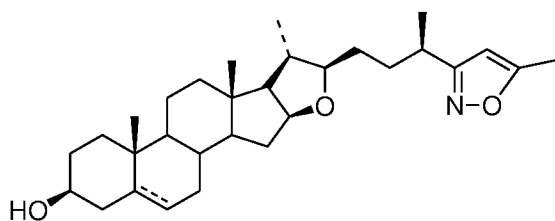
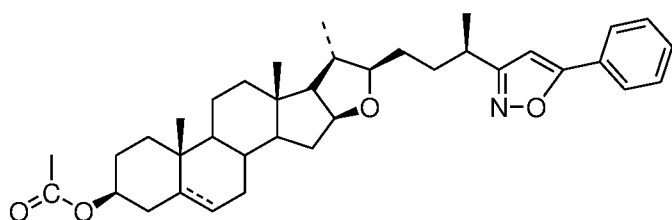
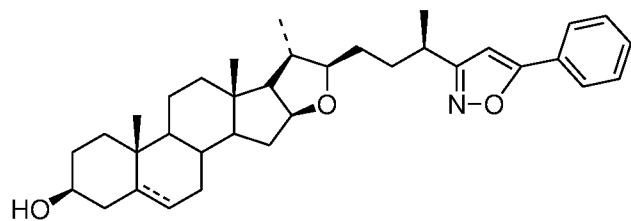


FIG. 2

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**7a-** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-methyl)isoxazole**7b-** (22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-methyl)isoxazole**7c-**(22 β ,25R)-3 β -hydroxymurost-5,6-dehydro-26-(5-methyl)isoxazole**7d-**(22 β ,25R)-3 β -hydroxymurost-5,6-dihydro-26-(5-methyl)isoxazole**8a-** (22 β ,25R)-3 β -acetoxymurost-5,6-dehydro-26-(5-phenyl)isoxazole**8b-**(22 β ,25R)-3 β -acetoxymurost-5,6-dihydro-26-(5-phenyl)isoxazole**FIG. 3 (continued....)**

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8c (22 β ,25R)-3 β -hydroxyfurost-5,6-dehydro-26-(5-phenyl isoxazole)

8d (22 β ,25R)-3 β -hydrooxyfurost-5,6-dehydro-26-(5-phenyl isoxazole)

FIG. 3

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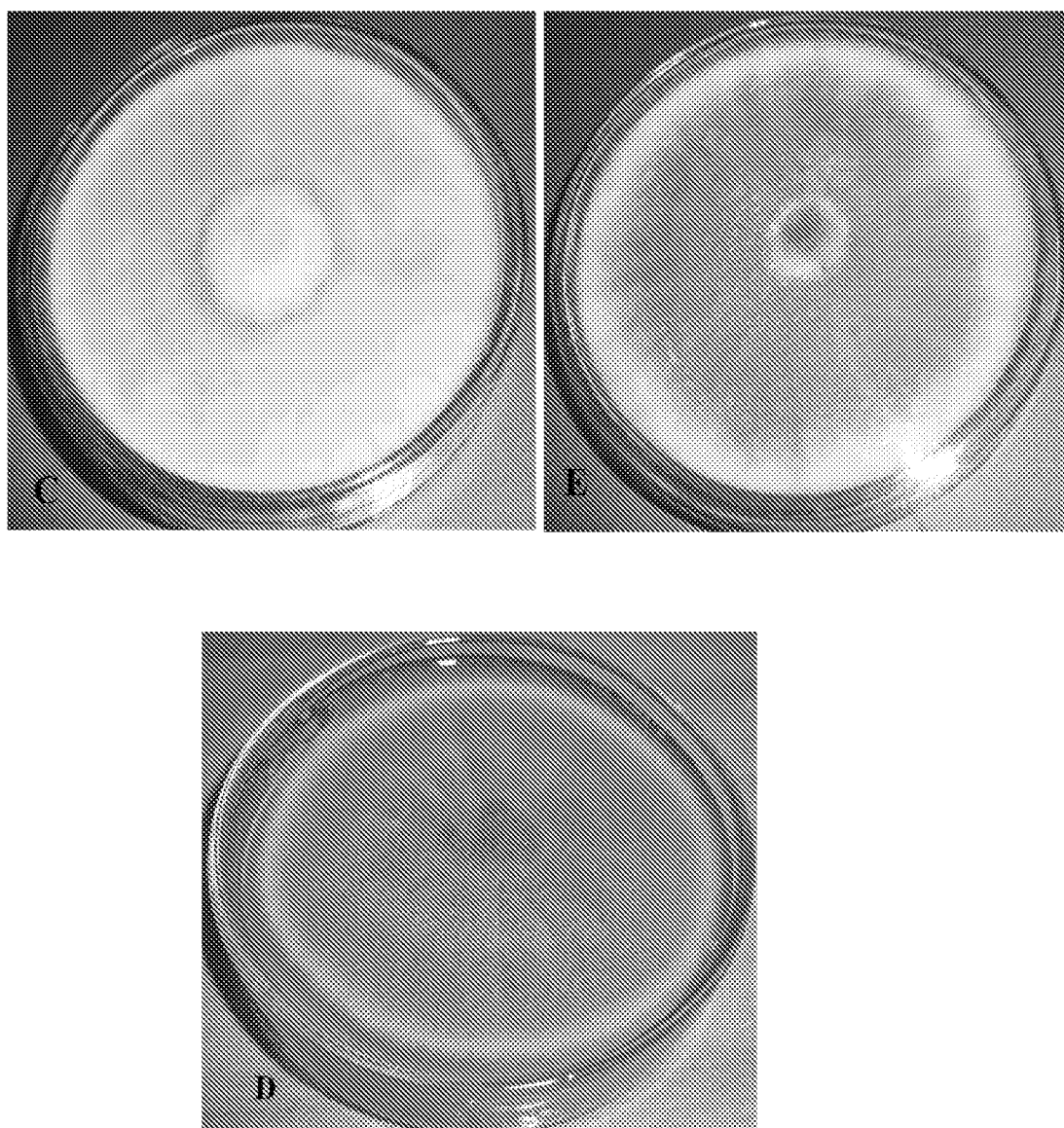


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2016/050062

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07J71/00 A61P31/10 A61K31/58
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)
C07J A61K A61P

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SINGH MONIKA ET AL: "Synthesis of diosgenin analogues as potential anti-inflammatory agents", JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, ELSEVIER SCIENCE LTD., OXFORD, GB, vol. 143, 9 May 2014 (2014-05-09), pages 323-333, XP029016112, ISSN: 0960-0760, DOI: 10.1016/J.JSBMB.2014.04.006	8
A	compounds 9,10,16 ----- -/--	1-7,9,10



Further documents are listed in the continuation of Box C.



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 application or patent 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

6 June 2016

Date of mailing of the international search report

15/06/2016

Name and mailing address of the ISA/

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Authorized officer

Johnson, Claire

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2016/050062

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAMID A A ET AL: "Synthesis of novel anticancer agents through opening of spiroacetal ring of diosgenin", STEROIDS, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 87, 12 June 2014 (2014-06-12), pages 108-118, XP029009616, ISSN: 0039-128X, DOI: 10.1016/J.STEROIDS.2014.05.025	8
A	compounds 7,8,14 -----	1-7,9,10
A	YANG CHONG-REN ET AL: "Antifungal activity of C-27 steroidal saponins", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 50, no. 5, 1 May 2006 (2006-05-01), pages 1710-1714, XP002602719, ISSN: 0066-4804, DOI: 10.1128/AAC.50.5.1710-1714.2006 cited in the application the whole document -----	1-10
A	US 2008/287662 A1 (WIESMAN ZEEV [IL]) 20 November 2008 (2008-11-20) paragraph [0062]; claim 1 -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IN2016/050062

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008287662 A1	20-11-2008	EP 1893022 A2	05-03-2008
		US 2008287662 A1	20-11-2008
		WO 2006137069 A2	28-12-2006
