No.5/57/2009-PI-I/III (Part)
Government of India
Ministry of Chemicals & Fertilizers
Department of Pharmaceuticals

Shastri Bhawan, New Delhi,
Dated 28th August, 2017.
4th Sept.

To

Pharmaexcil, FICCI, CII, PHDCCI,
ASSOCHAM, IPA, IDMA, BDMA,
FOPE, OPPI, ABLE & CIPI.

Subject : Reg.Production and Availability of Medicines to deal with Swine Flue (H1N1) and the Stock of Oselamivir API/Capsules in the Country.

Sir,

The undersigned is directed to say that the ICMR has developed technology for reducing dependence on imported shikimic acid (basic API) for manufacture of Oselamivir. A note on "Indigenous production of Shikimic Acid : a key starting material for manufacturing the drug Tamiflu" describing salient features of the technology received from ICMR is enclosed for perusal.

2. You are requested to disseminate the information amongst your members to explore possibilities to commercially adopt the technology developed by the Indian Council of Medical Research (ICMR) to reduce the dependence on imported shikinic acid.

(H.K. Mallick)
Under Secretary to the Govt. of India.
Tel : 23383392.

Encls : As Above.

Copy to : -

1. Consultant to JS (SP).
2. PS to DS (MKB).

Indigenous production of Shikimic Acid : a key starting material for manufacturing the drug Tamiflu.

Indian Council of Medical Research (ICMR), New Delhi is seeking potential agencies/ companies interested in **Indigenous production of Shikimic Acid : a key starting material for manufacturing the drug Tamiflu**

INTRODUCTION

In order to produce antiviral drug Tamiflu which reduces the severity of symptoms arising out of Avian Flu primarily caused by H5N1 and H1N1 viruses, ICMR took initiative to fund research projects aiming at producing shikimic acid (the starting material for the production of Tamiflu) indigenously.

SALIENT FEATURES OF THE TECHNOLOGY

Technology (Project I)

A massive screening of different bacteria resulted in the selection of two strains of *C. freundii i.e.* MH0711 and YRL11 as potent shikimic acid producers. The identification of these strains was confirmed by 16s RDNA sequencing. Initially the strains were producing 0.87 and 0.69 g/L of shikimic acid in a reported control medium. After subsequent process optimization, a successful enhancement in the yield could be achieved which resulted in the production of 14.57 and 12.76 g/L of shikimic acid in 72 and 60 h of incubation.

Further scale up and its optimization including fed batch studies using carbon and nitrogen source finally resulted in an approximate yield of 21±1 & 22±1 g/L of shikimic acid in 60 and 48 h incubation. These studies were carried out in a bioreactor of 30 L and subsequently in 300 L vessel size. Extraction and purification was attempted and has resulted in approximately 50% of the recovery yield. However, patented process of purification will be of help for maximum extraction of shikimic acid from the fermentation broth. Electrodialysis may be an important way to achieve this target. The extracted and purified shikimic acid was evaluated for its HPLC, UPLC, NMR, IR, LCMS and melting point analysis. Results obtained showed that the shikimic acid thus obtained is as pure as sigma grade which was used as a standard in the present investigation.

Technology (Project II)

a) It has been discovered that leaves of *Anacardium occidentale* (cashew) contain 2.5 to 4.6% shikimic acid on dry weight basis. The green leaves of this plant contain 15-24% solid matter . It has also been identified that leaves of two Indian plants (*Terminalia chebula* & *Dahlia brocata*) ciontaion more than 3% (w/w) shikimic acid. A procedure for extraction and purification of shikimic acid from leaf tissue and its analysis has been defined .

b) Information has also been generated on *Bacillus pumilusthat* converts quinic acid to shikimic acid and dehydroshikimic acid . Optimisation of the fermentation protocol may further enhance the shikimic acid yield.

Technology (Project III)

- The availability of shikimic acid in plants was explored in both natural occurring forms as well as the accumulation by use of broad spectrum herbicide (intervention experiments). The plants were selected from literature studies and collected from different localities. Different combinations of ethanol in water were tested for the extraction purpose and finally 10% ethanol in water (v/v) was found to be most economical method. Cold maceration and pressurized liquid extraction (PLE) methods were used and finally PLE methods was selected. LC-MS method was developed for the detection of shikimic acid in plant material. The collected material was analyzed for the presence of shikimic acid with the help of LC-MS. The isolation of shikimic acid was done with the help of LC-MS and the method was reproduced for economic isolation with column chromatography. The reverse phase and ion exchange column chromatography were undertaken. Ion exchange column chromatography was scaled up to pilot scale for commercial isolation.

- A total number of 90 samples were analyzed. Out of these, 26 plant samples were collected from different locations and analyzed for the presence of shikimic acid. The presence of shikimic acid was confirmed in 13 plant samples. Out of these plant samples, the most potential source was *Pinus roxburghii* with shikimic acid percentage of ~2%. The characteristics which make *Pinus* the most potential source include its wide availability, evergreen habit, sufficient production of biomass and wide distribution range.

- The second approach was to induce the accumulation of shikimic acid in the locally available wild plants especially herbs. The plant showed diverse response in terms of accumulation of shikimic acid over different time periods to the application of broad spectrum herbicide. The response varied from accumulation of shikimic acid up to detectable levels reaching to maximum accumulation, gradual decrease and even death in some cases.

- The plants which responded well to the application herbicide were *Cannabis sativa*, *Sonchus oleraceus*, *Bidens pilosa* and *Ageratum conyzoides*. The plant were sprayed with 5% Roundup concentrations. Accumulation of shikimic acid for the given concentration of 5% Roundup was maximum in *Ageratum conyzoides.* The plant was further tested with lower concentrations of 2.5% and 1% concentrations. However, 5% Roundup concentration was found to be optimum for maximum induction (1.25%) of shikimic acid accumulation.

Therefore, *Pinus roxburghii* with natural availability of shikimic acid to the concentration of ~2% and *Ageratum conzyoides* with herbicide induced accumulation of shikimic acid to the concentration of 1.25% were found to be the most potential source of shikimic acid in the region.