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MINI-REVIEW

Expanding horizons of shikimic acid

Recent progresses in production and its endless frontiers in application and market trends

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Abstract Shikimic acid is an industrially important chiral compound used as a key ingredient in formulation of drug Oseltamivir phosphate (Tamiflu) for the treatment of swine/avian flu. The high cost and limited availability of shikimic acid isolated from plants has detained the use of this valuable building block of the drug. It is a versatile compound having many characteristic properties for many synthetic reactions particularly in pharmaceuticals and cosmetic industries. By virtue of being a natural product, the relevant biochemical pathway in microorganisms can be harnessed into fermentation processes to produce shikimic acid. This is an excellent alternative for the sustainable and efficient production of shikimic acid over the tedious and cumbersome process of plant based extraction methods. Various strategies of shikimic acid production are reviewed and an account of comparison of their challenges, promises and restraint is presented. Furthermore, present review attempts to focus on the market trend of shikimic acid due to its high demand with particular emphasis laid on the pandemics of swine flu. This review not only covers the recent advances in shikimic acid production but also highlights the versatile applications and its market scenario. The concluding remarks and its potential as a commercial bulk chemical are discussed in the light of current research.

Keywords Shikimic acid · Tamiflu · Swine flu · Synthesis · Biochemical pathway · Market · Application

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Introduction

The diversified range of biosynthetic pathways in plants and microbes contributes significantly in providing an array of lead structures which are the pivotal basis of many industrially interesting chemicals. For many centuries, the medicinal properties of plants and microbes have fascinated the scientists all over the world. The ability to produce valuable products via biological processes, using such diverse microorganisms and plants is proving to be a versatile and promising synthesis route for a growing slate of end products. Because such processes rely on the fermentation of renewable feedstock, they have the potential to offer an environment friendly and economic alternative to conventional synthesis routes that are based on petroleum-based feed-stocks, which face supply and price pressures (John et al. 2006; Simmonds 2003). Thus, the chemical industries are now realizing the utility of biological systems involving fermentation technology to effectively mediate chemical conversion for the production of industrially important compounds. Due to this, global interest for chemical production is being directed towards the research and commercialization of several microbial fermentation technologies (Cameron and Chaplen 1997; Saxena et al. 2009). Several studies (Frost and Sullivan 2011; Mackay et al. 2009) estimate the role of biotechnological processes in the commercial production of different chemical products to be around $16 billion in 2009; however, this figure is forecasted to increase to $22 by 2013 (Table 1). In response to this, there is a significant increase of interest in producing chemicals like shikimic acid, succinic acid, xylitol and other industrially important compounds by fermentation focusing mainly on the environmental concern, industrial safety and sustainable use of renewable resources (Abbad-Andaloussi et al. 1995;

Table 1 Global market for the fermentation derived chemicals (Mackay et al. 2009)

Chemical	2009 $ millions	2013 $ millions
Amino acids	5,140	7,821
Enzymes	3,200	4,900
Organic acids (lactic acid 20 %)	2,651	4,036
Vitamins and related compounds	2,397	2,286
Antibiotics	1,800	2,600
Xanthan	443	708
Total	15,901	22,351

Biebl 2001; Chotani et al. 2000; Dharmadi et al. 2006; Lee et al. 2001; Nemeth et al. 2003; Saxena et al. 2009). On account of this, shikimic acid is the valuable chemical which has attracted worldwide attention due to its characteristic pharmaceutical application as the only precursor for the formulation of drug against swine flu which has recently threatened the world. Earlier, shikimic acid was not considered as such an important chemical but the high demand and insufficient supply from plant source during the pandemic of swine flu has made this chemical utmost important.

Shikimic acid (3, 4, 5, tri hydroxy cyclohexene carboxylic acid) is an industrially important chiral compound that acts as a precursor in the synthesis of many chemical substances like anti-influenza drug Oseltamivir phosphate (Tamiflu) (Kancharla et al. 2009). Shikimic acid was first isolated and identified in 1885 by Eijkman from the fruit of the Japanese plant *Illicium religiosum* and the structure of shikimic acid (Fig. 1) was elucidated later after 50 years (Enrich et al. 2008). It is a key metabolic intermediate of the aromatic amino acid biosynthesis pathway found in microbes and plants (Sprenger 2006). A complete carbocyclic alignment of the chirality exhibited by shikimic acid has allured attention as the basis for synthesis of a large combinative chemical library (Knop et al. 2001). In the present decade, shikimate research has gained momentum due to concerns over outbreak of swine/avian flu which has elevated the demand of this important biomolecule. Ultimately, there was the hike in the market price, and it was raised to approximately $1,000/kg at the time of pandemic of swine flu in 2009 (Saxena et al. 2012). Shikimic acid serves as the starting material for the synthesis of drug Tamiflu (neuraminidase inhibitor), which is used in the treatment and prophylaxis of both influenza A and influenza B viruses. Thus, it acts as an important compound against swine flu. With the view of drastic effects of flu pandemic (Saxena et al. 2012), it has been estimated that in case of pandemic of swine/avian flu the present capacity of Tamiflu could be insufficient to protect the large affected population. Thus, maintaining stockpiles of effective drugs are necessary for managing such major outbreak.

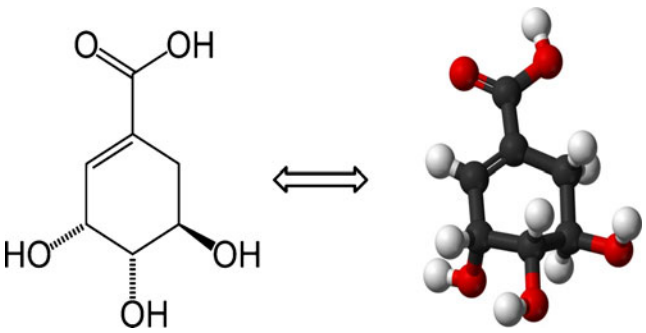

Fig. 1 Chemical structure and 3D model of shikimic acid

Shikimic acid has been traditionally considered as 'specialty chemical' is now undergoing transition into 'commodity chemical' (Frost et al. 2001). In recent years, shikimic acid has attracted the attention because of its additional industrial and biotechnological applications other than those in the pharmaceutical sectors of drugs. It finds many applications in the formulation of a variety of industrially important chemical products such as aromatic amino acids, indole and its derivatives, alkaloids and many other aromatic metabolites (Bochkove et al. 2011). Currently, it was also reported that shikimic acid has an innovative and multifunctional roles such as in dermo-cosmetic preparations, anti-enzymatic activity and an exfoliating agent for stratum corneum (Guglielmini 2010).

Majority of commercial synthesis of shikimic acid is from Chinese plant star anise but it is expensive and cumbersome including number of drawbacks. In this context, fermentation procedure using microorganism proved to be more efficient and viable alternative source to achieve large scale production of this expensive chemical. Several metabolic and genetic manipulations were conducted on different microorganism's mainly *Esherichia coli* to obtain shikimic acid. The chemical synthesis of shikimic acid is also known, but as per to environmental aspects not commercially viable.

Realizing the importance of shikimic acid, this article displays a light on the current scenario of shikimic acid production. Moreover, it is giving prominence to the applications and the market studies of shikimic acid which makes it more attractive and unconventional. Different strategies developed for enhancing the production of shikimic acid are discussed in along with the biochemical pathway. Its applications are reviewed by keeping its significance in drug formulation. It highlights the market opportunities of shikimic acid for other industrial sectors apart from pharmaceuticals. Furthermore, the market analysis for the pricing of shikimic acid is also reviewed in correlation with swine flu pandemics which is mainly responsible for the fluctuating prices. The concluding remarks and its potential as a commercial bulk chemical are discussed in the light of the current research and demand worldwide.

Current status of shikimic acid market in correlation to swine flu pandemics

Pandemics of swine flu

Swine influenza has emerged as the primary public health concern of the 21st century. It is a respiratory disease caused by influenza A H1N1 virus. The current pandemic of swine flu is most probably due to a mutation — more specifically, a re-assortment of four known strains of influenza A virus subtype H1N1. Antigenic variation of influenza viruses while circulating in the population is an important factor leading to difficulties in controlling influenza by vaccination. A highly lethal but non-human to human-transferable influenza A subtype, H1N1 caused swine flu that emerged and raged through Southeast Asian countries, Egypt and other countries, after the preparation of the world for the 2008 pandemic influenza, i.e., H5N1, bird flu (Lagacé-Wiens et al. 2010). Due to the rapid global spread of the H1N1 strain, the World Health Organization (WHO) announced a global pandemic alert to phase 6 on June 11, 2009 (Saxena et al. 2012; WHO 2009). In response to the global effect of swine flu and its impact on humans, extensive investigations are being undertaken for its ultimate cure. In this context, Tamiflu is the only available drug used in prophylaxis and is made from the compound shikimic acid (Saxena et al. 2012).

Epidemics rapidly spread to many countries without any respect to the national boundaries and can cause morbidity and mortality as well as interruptions and loss in trade, travel and tourism. The threat of human infections and the possibility of an influenza epidemic will remain as long as the outbreaks in poultry persist (Busquets et al. 2010). Thus, there will be a swinging market of shikimic acid depending on the importance and urgency of its demand for the manufacturing of drug Tamiflu.

Current status of shikimic acid market

Earlier, the market for shikimic acid was quite small. However, it is now emerging as a large volume commodity chemical. Recent applications of shikimic acid as building block of the drug Tamiflu has led to the strong market need for the production of this bulk chemical preferably by biological route. In biological routes the main emphasis is on fermentation based synthesis because of its ease in handling, efficiency and higher yield can be obtained in short time period. In recent years, demand for Tamiflu increased and drug company Roche could not meet demand due to a shortage of plant-derived shikimic acid (ETC group 2012). Because of the shortfall in botanically sourced shikimic acid, researchers increased efforts and attempted to produce it from alternative route of fermentation. Though, isolation from Chinese Star anise fruit was the principal source of commercial shikimic acid.

In 2009, market situation of shikimic acid changed significantly when WHO declared the global pandemic of swine/avian flu and countries started stockpiling of the drug to fight against this deadly disease (Schnitzler and Schnitzler 2009). Generally, shikimic acid was available at the price of $40/kg in the market (Awang and Blumenthal 2006; ETC group 2012; Pollack 2005). But due to occurrence swine flu outbreaks in the world, the price of shikimic acid soared to $400–500 per kilogram from just $40 (ETC group 2012; Pollack 2005). However, shikimic acid price is volatile with reports of high prices in the range of $700/kg (MacKay et al. 2009). China covers 80–90 % production of star anise, which are mainly grown in Guanxi and Yunnan province and estimated to account for 66 % of Star anise harvest is used to make Tamiflu (Wang Guanqun 2010; Xinhua 2010). The extraction and purification of shikimic acid from Star anise is expensive. Around 30 kg of Star anise is required to yield 1 kg of shikimic acid enough to treat one person. And the natural calamities like drought and floods affects the yield and restrict the supply and availability to the chemists and ultimately there is the increased price of shikimic acid. With the heightened threat of swine flu pandemics in the year 2009 the demand for Tamiflu increased and it reached to $2.9 billion (Doherty 2009; Roche 2009). However, the worldwide sale of this antiviral drug fluctuates as in 2011 sales reached only $406 million (Doherty 2009; ETC group 2012; Roche 2009).

The major raw material is glucose in shikimic acid fermentation (Frost et al. 2002; Rangachaari 2010). More than 30,000,000 tons of glucose is produced annually worldwide, and it is sold at a price of about $0.39/kg (Songa and Lee 2006). Assuming the shikimic acid yield of 51.8 % (w/w) on glucose (Escalante et al. 2010), the raw material cost in the bioprocess is then $0.75/kg. It is thus clear that fermentative production of shikimic acid from renewable resources can compete with the plant based extraction process. More importantly, sudden high demand and ever increasing environmental concerns are urging us to replace the plant based extraction processes with fermentation processes. Renewable resources are more than abundant, currently being estimated to be 170 billion tons/year, of which only 3.5 % is being utilized.

Production aspects of shikimic acid: an overview

Chemical synthesis of shikimic acid

Shikimic acid was earlier successfully manufactured using basis Diels–Alder reaction with a very low yield ≥15 % (Bochkove et al. 2011; Bruce and Bohm 1965; Ghosh et al. 2012; Mc Crindle et al. 1960; Smissman et al. 1959). Later, attempts were made to increase the efficiency of Diels–Alder reaction which resulted in an increase of 29 % which with

further advancement in research rose to 55 % yield (Fig. 2) of the product (Bochkove et al. 2011; Ghosh et al. 2012; Grewe and Hinrichs 1964; Koreeda and Ciufolini 1982; Koreeda et al. 1990). Carbohydrates are naturally abundant and inherently rich in chiral centers and have emerged over the years as a important base material for chiral compounds. Enantiomerically, pure shikimic acid and its enantiomers have been reported to be synthesize from D-arabinose, D-mannose and D-ribose (Fig. 3) (Bestmann and Heid 1971; Dangschat and Fischer 1950; Fleet et al. 1984; Jiang et al. 1999; Kancharla et al. 2009; Li and Frost 1999). Also enantio-convergent synthesis of shikimic acid has been reported by employing the palladium mediated elimination reaction as the key step which is due to biased framework and thermal instability of tricyclic substrate (Yoshida and Ogasawara 2000). Shikimic acid can also be synthesized from quinic acid and its derivative by chemical transformation (Ghosh et al. 2012; Hasegawa et al. 1957; Yoshida and Hasegawa 1957). Other than that, efficient synthesis of shikimic acid and its epimers with high yield of 32 and 52 % has been reported which is due to its advantage of adaptability that can be used to serve as building block for glycomimetics (Griesbeck et al. 2007; Grim et al. 2011).

Although it is possible to generate shikimic acid by these chemical methods, these are either capital-intensive and/or generate waste streams containing environmental pollutants thus are not commercially viable. Henceforth, isolation from Chinese star anise fruit remains the principal source of commercial shikimic acid (Raghavendra et al. 2009). In recent years, some attractive biological processes have been developed for the production of high quality commercial shikimic acid derived from renewable resource at low cost. In this direction, fermentation process using microorganisms are interesting entities as these produce shikimic acid as the byproduct of their metabolism. Chemical synthesis of shikimic acid and its derivatives is further reviewed elsewhere (Bochkove et al. 2011; Bruce and Bohm 1965; Ghosh et al. 2012).

Extraction from plants: a traditional strategy

Shikimic acid is the biosynthetic intermediate in the aromatic amino acid pathway. The widespread availability of shikimic acid pathway in plants has made its ease in the extraction of this valuable biomolecule for its wider range of applications. It has been found to occur in many tissues of different plants but the content accumulation depends on the metabolic processes. The distribution and quantitative estimation of shikimic acid in different plants was first done by Hasegava (Gaitonde and Gordon 1958; Hasegawa et al. 1957) and later different other techniques were devised by others (Gaitonde and Gordon 1958; Millican 1963; Saslaw and Waravdekar 1960; Yoshida and Hasegawa 1957). The concentration of shikimic acid in different plants organs decreases or remains constant for some time depending on the metabolic processes leading to the synthesis of aromatic compounds (Bochkove et al. 2011). A high percent content of 24 % shikimic acid was discovered in the *I. religiosum* fruits but the isolation yield is low 7–10 % (Adams et al. 1996; Bochkove et al. 2011). Extraction of shikimic acid from G*ingko biloba* leaves with a yield of 2. 3 % has been described (Usuki et al. 2011). Subsequently, it was also reported in the seeds of *Liquidambar styraciflua* which contain 2.4–3.7 % shikimic acid (Enrich et al. 2008). Evidence of shikimic acid was also found *Eucalyptus*, *Pinus*, *Picea* and *Gingko* species plants (Hillis 1964; Hillis and Carle

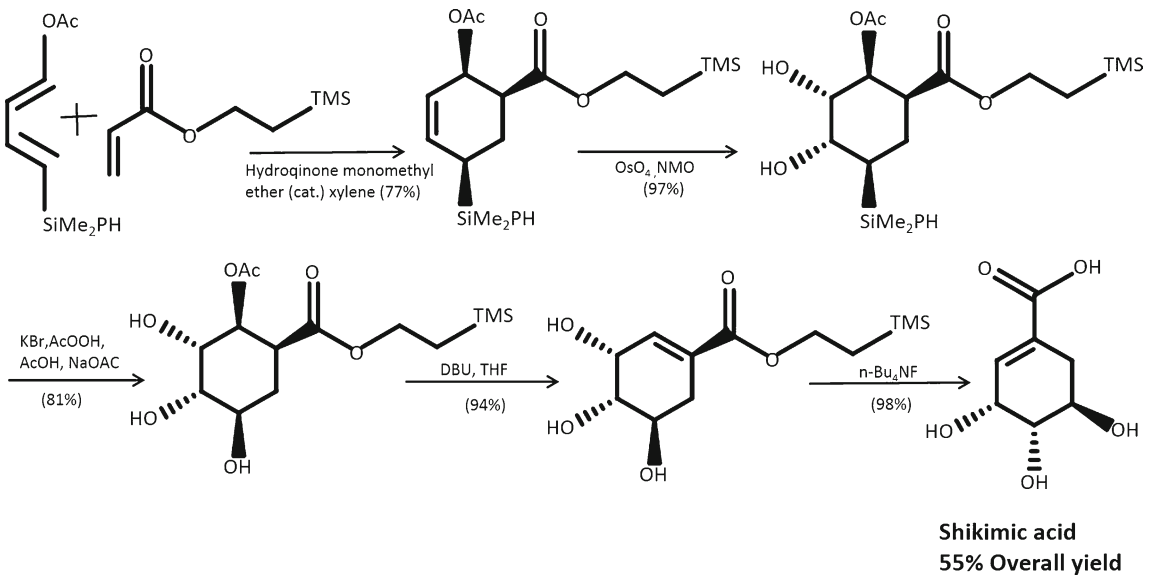

Fig. 2 Koreeda's second-generation synthesis of shikimic acid (Ambhiakar 2005; Koreeda et al. 1990)

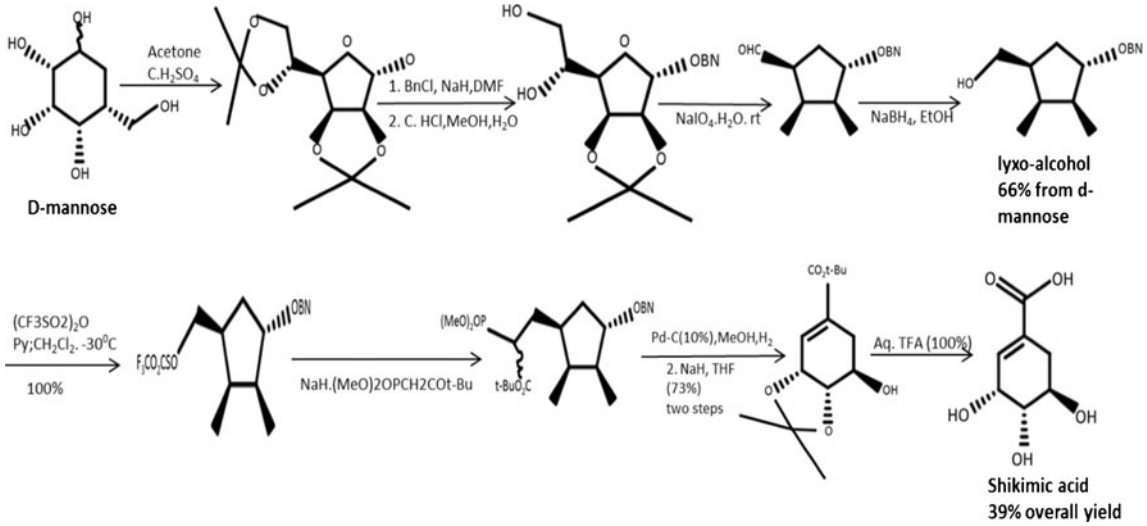

Fig. 3 Chiral synthesis of shikimic acid from carbohydrates (Ambhiakar 2005; Fleet et al. 1984)

1958; Lei et al. 2009; Qin et al. 2011; Yun et al. 2012). Isolation of shikimic acid from Chinese Star anise (*Illicium vernum*) has been explained by Richard Payne employing ion exchange chromatography where anion exchange resin Amberlite IRA 400 acetate form was used to obtain pure shikimic acid with a recovery yield of 5–7 % (Payne and Edmonds 2005). A rapid and efficient extraction procedure of hot water extraction at temperature range of 30–200 °C has been developed for various particle size, water flow rate and contact time from Star anise (Ohira et al. 2009). The extraction of shikimic acid coupled to dilute acid pretreatment was also reported from sweetgum (*Liquidambar styraciflua*) (Enrich et al. 2008; Martin et al. 2010). Extraction of shikimic acid from pine needles of *Pinus elliottii* by decompressing inner ebullition was studied where the extracting proportion of shikimic acid from dry pine needles of *Pinus elliottii* was 1.51 % (Xie et al. 2010; Yun et al. 2012). Separation of shikimic acid from pine needles followed by subsequent evaporation, column adsorption/desorption and crystallization processes was also described which resulted in shikimic acid crystals with a purity of over 98 %. A total recovery of approximately 85 % is achieved (Payne and Edmonds 2011; Sui 2008). Recently, shikimic acid as a major compound from *Ludwigia alternifolia* has been isolated using vacuum liquid chromatography over RP C18 silica gel column (Rao and Mergur 2012). Accumulation of shikimic acid by treatment of glyphosate in the plants *Illicium*, *Zea*, *Lollium* has been explained for the extraction purpose (Payne and Edmonds 2011; Pavlovic et al. 2011; Rao and Mergur 2012). Several other methods for the extraction of shikimic acid from plants have been reported employing different strategies. However, obtaining pure shikimic acid from plants is really a very tiresome and expensive process. As a plant extract it contains many interfering impurities which have to be removed and thus requires extensive procedures to yield high purity shikimic acid. Beside this, still commercial synthesis of shikimic acid is mainly procured from Star anise.

Microbial production of shikimic acid: an efficient approach with high yield

The microbial production of shikimic acid using fermentation technology is a promising and attractive alternative for traditional chemical procedure or the plants based extraction methods. Shikimate pathway has been characterized in plants by Herrmann and in bacteria by Pittard (Herrmann 1995; Kramer et al. 2003; Pittard 1996). The system has been most thoroughly studied in *E. coli* (Kramer et al. 2003; Pittard 1996). Therefore, microorganisms can be utilized for the production of this important biomolecule which is itself the precursor of many other compounds in the pathway. Till now, only metabolically engineered or mutated bacteria are employed for the higher production of shikimic acid. Mainly the two different approaches has been attempted for the fermentative production of shikimic acid — blocking the aromatic amino acid pathway at the step which eliminates the enzyme responsible for further conversion of shikimic acid to other compound either by genetic engineering involving only *E. coli* or random mutagenesis of bacteria *Citrobacter* and *Bacillus* (Iomantas et al. 2002; Shirai et al. 2001). Fermentative production of shikimic acid was most successfully carried out only from rationally designed *Escherichia coli* strains, but production has also been reported from other bacteria. Different metabolic strategies have been reported in the strain of *E. coli* for achieving higher production of shikimic acid (Johansson 2006; Johansson and Liden 2006; Johansson et al. 2005; Knop et al. 2001). Under glucose-rich conditions, hydroaromatic equilibration is inhibited by the overexpression of transketolase leading to the synthesis of

52 g/l shikimic acid with a yield of 18 % (Knop et al. 2001). Inactivation of PTS and *pyk f can* further increase the PEP availability for shikimic acid production which resulted in high yield of 0.48 g/g on glucose (Escalante et al. 2010).

Also, the expression of non PTS glucose transporters like glucose facilitators, glucose kinase along with over expression of transketolase was studied to increase the amount of shikimic acid to 71 g/l (Chandran et al. 2003; Gibson et al. 2001). In recombinant *E. coli*, the shikimic acid concentration could be increased during the exponential phase of growth to 87 g/l with a maximum productivity of 5.2 g/l/h by the supplementation of yeast extract in the medium (Chandran et al. 2003). Exploration of the effect of carbon sources on the metabolic capacity for shikimic acid production in shikimate kinase deficient *E. coli* strain reported the highest yield of 0.36 g/g for glycerol, whereas the highest shikimic acid production rate of 0.33 g/l/h was obtained for glucose (Ahn et al. 2008). The study suggested that the type of carbon source affects shikimic acid productivity. The problem of simultaneous production of quinic acid during the biosynthesis of shikimic acid due to microbe catalyzed equilibration of initially synthesized shikimic acid can be overcome by the high titers of shikimic acid (Knop et al. 2001). Apart from recombinant or genetically modified bacteria some mutations upon bacteria like *B. subtilis* and *Citrobacter* has been reported for achieving higher production of shikimic acid which are made shikimate kinase deficient either by exposure to certain chemicals (EMS or MMS) or UV rays. The strain of *B. subtilis* lacking aro l gene was reported to produce 8.5 g/l shikimic acid as the byproduct whereas a chemical mutant of *C. freundii* produces 10 g/l of shikimic acid (Iomantas et al. 2002; Shirai et al. 2001). Recently, the use of herbicide namely glyphosate, an EPSP synthase inhibitor in the production medium blocks the further consumption in the pathway and enhances the production of shikimic acid (Bogosian and Frantz 2011; Rangachaari 2010). In addition microbial biotransformation has also been reported for shikimic acid production. The ability of the bacteria *Gluconobacter oxydans* to convert quinic acid to shikimic acid is been utilized for obtaining shikimic acid. A two-step scheme for biotransformation of quinic acid to shikimic acid has been published (Ghosh et al. 2012; Griesbeck et al. 2007; Grim et al. 2011). This scheme involves an oxidative fermentation with *G. oxydans* which essentially converts quinic acid to dehydroshikimic acid in the culture medium which is subsequently converted to shikimic acid in an NADPH-dependent reaction catalyzed by shikimic acid dehydrogenase (Adachi et al. 2003, 2006).

At present, the microbial routes are still facing competition from the plant based extraction procedure. Though, the microbial processes are proprietary, gaining more and more importance due to its characteristic sustainability and efficiency. Along with that use of renewable resources, ease, economics, rapid procedure are the other advantages of this fermentation process over others. In this context, we would like to mention our studies on shikimic acid wherein we have achieved a successful production of shikimic acid from a wild type bacteria using simple fermentation technology (Rawat et al. 2013; Saxena et al. 2012). However, the economy of a process will largely determine its fate on the market. This drives continuous innovations and technology development for the fermentative production of shikimic acid.

Shikimic acid pathway

It is well known that shikimate pathway is widely distributed in plants and microorganism. Shikimic acid plays a key role being an intermediate of the pathway to produce essential aromatic amino acids and many other compounds which are growth associated and also of industry interest. The shikimate pathway (Fig. 4) has been studied and previously reviewed (Herrmann and Weaver 1999; Maeda and Dudareva 2012; Tzin and Galili 2010; Wilson et al. 1998). The biosynthesis of shikimate pathway initiates with the condensation of phosphoenolpyruvate (PEP; from the glycolytic pathway) and of erythrose 4-phosphate (Ery4P; from the pentose phosphate pathway) to form DAHP (3-deoxy-D-*arabino*-heptulosonate 7-phosphate) (Arcuri et al. 2010; Gibson and Pittard 1968; Kramer et al. 2003; Wilson et al. 1998). Thus, these two compounds of glycolytic and pentose phosphate pathways are the key precursors and play an important role in the regulation of the carbon flux into the shikimate pathway (Kramer et al. 2003; Iomantas et al. 2002). The first step is aldol condensation catalyzed by metal dependent DAHP synthase (Dewick 1995; Gleason and Chollet 2011). The shikimate pathway proceeds further from DAHP via 3-dehydroquinate and 3-dehydroshikimate to shikimate. Further, shikimate 3-phosphate is formed by ATP-dependent phosphorylation, which accepts a second molecule of PEP to form 5-enolpyruvylshikimate 3-phosphate. In the last step of this pathway, chorismate is formed (Gleason and Chollet 2011; Knop et al. 2001). All the intermediates of the pathway can be considered as branch point to serve as the precursor of other metabolic pathways (Dewick 1995; Gleason and Chollet 2011; Knaggs 2001, 2003; Wilson et al. 1998). The activity of DAHP synthase gene in microorganism is regulated by allosteric feedback inhibition by different aromatic amino acid (Dell and Frost 1993; Gleason and Chollet 2011; Romagni 2009). In bacteria, the expression of DAHP synthase is regulated by direct binding of the main transcriptional factors with aromatic amino acids. In fungi, the transcriptional activators (Gcn4p) get induced under aromatic amino acids starvation and modulate the expression of DAHP synthase gene. However, in plants limited information is available for the evidence of this gene expression. In microorganisms, the

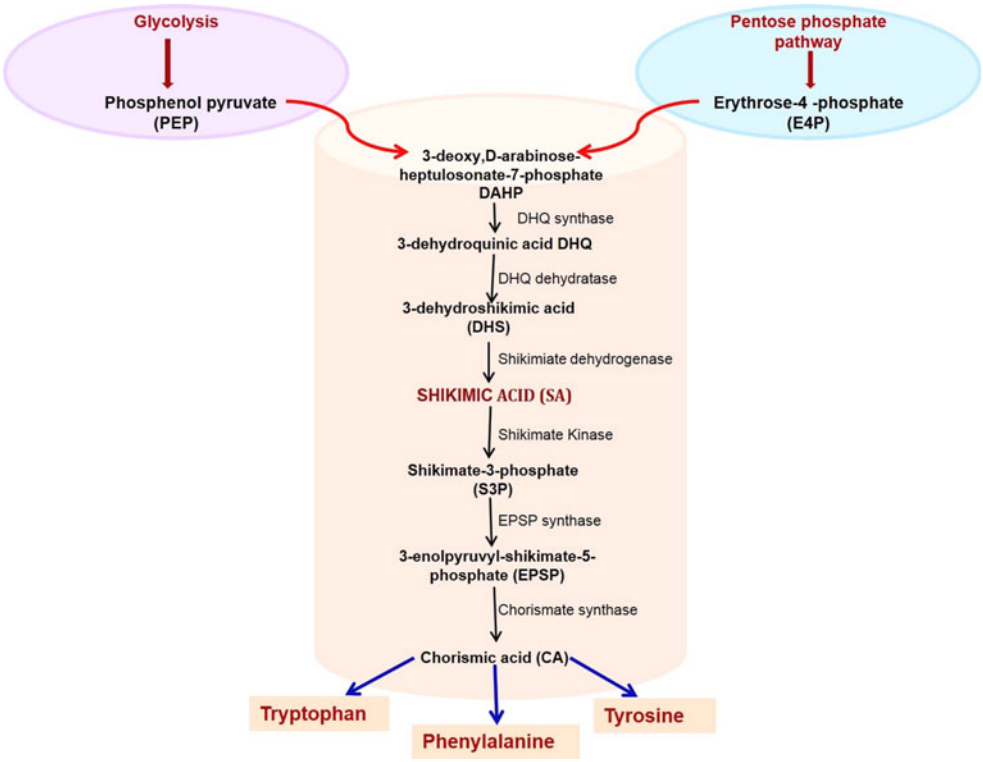

Fig. 4 Shikimic acid pathway

enzyme activity of DHQ synthase becomes limiting due to allosteric feedback resistant DAHP which further causes the accumulation of DAHP. However, DHQ synthase and shikimate kinase are rate-limiting enzymes of the pathway (Kloosterman et al. 2003; Nie et al. 2009).

Applications of shikimic acid: versatility seeking place in market

Shikimic acid also has a plethora of interesting industrial application. As discussed previously, in pharmaceuticals shikimic acid acts as starting material for the synthesis of drug Oseltamivir phosphate (Tamiflu) (Knop et al. 2001; Nie et al. 2009; Pollack 2005; Raghavendra et al. 2009; Knop et al. 2001). Current industrial synthesis of Oseltamivir phosphate (Fig. 5) has been developed by the chemists at Gilead Sciences, Inc. and F. Hoffman-La Roche. Among all the reported synthetic methods, La Roche's method seems to have been the best one for industrial large-scale preparation of Tamiflu, but there are some drawbacks associated with this method like the long synthetic route and the relatively low total yield. In this context, a short and practical synthesis of

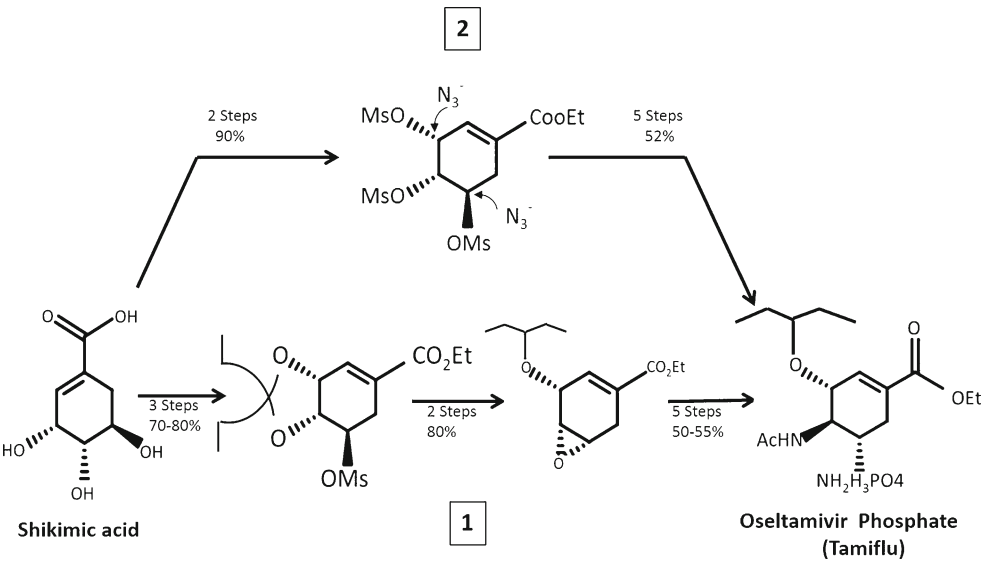

Fig. 5 Synthesis of Tamiflu: (1) current industrial synthesis of Oseltamivir phosphate (GILEAD: Rohloff et al. 1998, ROCHE; Federspiel et al. 1999); (2) short route synthesis of Tamiflu (Nie et al. 2009)

Oseltamivir Phosphate (Tamiflu) from (−)-Shikimic acid has been reported (Nie et al. 2009) which constitute overall eight steps and high yield (47 %) (Fig. 5). Other merits include inexpensive reagents, mildness of the reaction conditions, and ease of manipulation of every step. Shikimic acid is also considered as a useful additive in hair growth products due to the interaction in the biochemistry of serotonin and melatonin which is recently reported to be involved in treatment of alopecia (Guglielmini 2010; Sakaguchi et al. 2004).

Sinerga Laboratories has investigated the cosmetic uses for shikimic acid and launched it commercially under the name Verochic (Guglielmini 2010). Verochic is particularly suitable for deodorants as roll-on, spray and cream formulations. It has a unique anti-enzymatic activity on lipase which inhibits degradation of triglycerides into free fatty acids (Armesto et al. 2003; Guglielmini 2010), and thus act as sebum controller which in turn reduces and controls body odours and provide the deodorizing effect. Verochic could be also used as a modulator of skin hyperkeratosis since it reduces thickness of the outer layer of epidermis along with antimicrobial properties. Thus, due to its antibacterial and exfoliating properties, it could be also used in anti-acne creams and emulsions in controlling follicular hyperkeratosis and bacterial proliferation in acne problems (Guglielmini 2010). It could be considered also for anti-dandruff shampoos and lotions. Furthermore, it can be converted to a series of fine chemicals such as phenol, benzoic acid, protocatechuic acid, and gallic acid through aromatization (Mackay et al. 2009).

Considering the versatile nature of shikimic acid and its expanding horizons of applications in different areas, it is very clear that this bio-feedstock deserves further research and application as a renewable and reliable resource for modern chemical and pharmaceutical industries. The ever-increasing demand for this chemical definitely indicates its potential as a strong entity in the market, and sooner or later it is expected to serve as the basis for other more industrially important chemicals.

Conclusion

It is well realized that shikimic acid from biotechnology shows great promise in relieving the dependence of modern pharmaceutical industry and society on a diminishing and fragile supply from botanically sourced shikimic acid. Considering these findings altogether, the exploitation of microbes via bioprocess engineering is the prompt way for the fermentative production of shikimic acid. The direct fermentation of glucose allows the use of a renewable feed stock in contrast to expensive and cumbersome routes that are dependent on plants or chemical procedures. Today, the importance of shikimic acid lies predominantly in its versatile application in pharmaceuticals as the key ingredient for the commercial synthesis of drug Oseltamivir phosphate (Tamiflu). Shikimic acid can be synthesized chemically but allegedly not commercially viable. In fact, the chemical synthesis of Oseltamivir may entirely elude the need for shikimic acid; however, because of environmental concerns it is not commercially used. Thus, availability of low-cost shikimic acid will be the key to competitiveness in the market, and reduced cost will be the main factor in order for the process to become commercially viable.

Metabolic engineering helps to solve the define problems associated with the production of this highly valued compound. It expands the range of chemical process solutions and enables improved cost effective options based on fermentation routes. The production of shikimic acid proves to be a good example of a successful approach of rationale strain design by metabolic pathway engineering for the efficient and sustainable production with high yield. The story of shikimic acid represents a benchmark in the realization of the importance of any compound that is preliminarily not so important and suddenly gaining global demand and value due to some serious reasons like swine flu pandemics. It is well known that epidemics of swine flu have hit the world many times in the past, and every time this occurs, it appears with a newly evolved virus such that proper medical precautions and treatment are insufficient to cure it. The shortfall in the availability of shikimic acid at such moments is crucial and dangerous, too. The current fermentation-based production proves to be a successful process which is efficient and low cost and can meet the high demand for continuous supply throughout the year. However, further work needs to be done in order to develop a more enhanced biological procedure, robust microorganism, and efficient bioreactor designs for the process to become economically viable. Thus, for the accomplishment of this vision, we need more expertise in research and development to increase motivation, efficiency, and sustainability for the production of high valued compounds.

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