

**REVIEW ARTICLE**

**Vanilla- Natural Vs Artificial: A Review**

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**ABSTRACT:**

Natural and artificial flavours are identical. Natural flavours made by extracting chemicals from natural ingredients, artificial flavours are made by creating the same chemical compositions synthetically. The most popular flavouring compound - Vanilla, can find its application in food and beverage industry, perfume and pharmaceutical industries. This review features natural vanilla flavouring from the vanilla bean. The extraction process, chemical constituents and health benefits are emphasised. Culinary uses of vanilla flavouring are analysed. Production of artificial/synthetic vanillin and its health impact is also highlighted. Food and Drug Administration rules have been discussed. From this context consumer awareness and understanding are significant towards the difference between artificial and natural vanilla flavouring and their associated benefits, which help them to make the right decisions for their well being.

**KEYWORDS:** Flavour, Natural Vanilla, Artificial Vanillin, Culinary Uses, Health benefits and Impact, Food and Drug Administration.

**INTRODUCTION:**

Flavour plays a key role in the acceptance or rejection of food<sup>1</sup>. The most popular flavouring compound - Vanilla, can find its application in food and beverage industry, perfume and pharmaceutical industries<sup>2,3</sup>. 'Vanilla' is derived from Spanish, meaning sheath or pod and 'illa', conveys little (i.e.) a vine yielding small pods.

Vanilla pods are macerated and percolated in a solution of ethanol and water and thus vanilla extract is obtained. The compounds, oil and aroma in vanilla beans are extracted from the plant genus *planifolia*. Vanilla flavour is an indispensable ingredient in many bakery and confectionary products, custards, ice creams, and puddings<sup>4</sup>.

The objectives of this article are to differentiate between natural as opposed to artificial vanilla flavour additive. Culinary uses of vanilla flavour are discussed and also chemical additives in synthetic vanillin are listed out in this review.

Acceptable Daily Intake (ADI) and Food and Drug Administration rules are mentioned. Also to high light whether *vanillin* extracted from nature is safer or artificial made vanillin is safer.

**Natural vanilla flavourings:**

Natural Vanilla flavour is obtained from total of 110 species of plant genus *Vanilla* which belongs to the family Orchidaceous, a tropical hiking orchid. *Vanilla planifolia* and *Vanilla tahitensis* are two species from this genus have been approved in most countries, however because of its pod quality and yield, *Vanillus planifolia* is widely recommended. The flavour and aroma of vanilla extract is due to the presence of vanillin (4-hydroxy-3-methoxybenzaldehyde). The species of *Vanilla planifolia* having a highest vanillin content comparatively with the species *Vanill tahitensi*<sup>5</sup>

**Cultivation of Vanilla:**

Indonesia and Madagascar are the largest producers of *V. planifolia*. The influx of Spaniards in Mexico and the discovery of artificial pollination techniques, *V. planifolia* cultivation is possible in many tropical climates<sup>6</sup>.

USA, Germany, France and Netherland are the main consumers of vanilla. The demand for natural vanillin increases in the international market year after year. Vanilla is originated from South East Mexico but above

90 per cent of vanilla production comes from the Indian Ocean Island nations, Indonesia and Madagascar<sup>7</sup>.

India entered the international vanilla market during a crisis due to shortage of supply from Madagascar and resulting price rise. Now in India, vanilla cultivation has become a major activity for farmers in Tamil Nadu, Karnataka and Kerala<sup>4, 8 and 9</sup>.

**Types of vanilla beans:**

Bourbon-Madagascar, Mexican and Tahitian are the 3 major types of vanilla. The Bourbon-Madagascar vanilla is featured with thin pod, a rich and sweetest flavour. Mexican vanilla tastes smooth and rich, while Tahitian vanilla has the thickest and darkest-coloured pod that's aromatic with less flavour comparatively<sup>10</sup>.

**Bourbon Vanilla:**

Bourbon vanilla is long and slim pod, with a rich and sweet flavour. It has a thick, strong vanilla notes and contain a large quantity of tiny seeds. Bourbon Vanilla is a generic term for *Vanilla planifolia*<sup>10 and 11</sup>.

**Mexican vanilla:**

The flavour of Mexican vanilla has a mellower, smooth, quality and a spicy, woody fragrance. Their robust flavour is great for rich baked goods, sauces, ice cream, sweet breads, custard, cheesecake and other desserts<sup>12</sup>.

**Tahitian vanilla:**

Tahitian vanilla is rare species from France Polynesia. These beans are more subtle than Madagascar beans comparatively. Rather than being sweet and strong, Tahitian vanilla beans are usually blended with ice creams, custards and fruit-based desserts. Tahitian vanilla beans are used in cold foods. The essential oil from Tahitian vanilla beans is blended into perfumes and soaps and imparted its flavour. Tahitian vanilla beans are so subtle when added to body fragrances<sup>13</sup>.



A. Bourbon Vanilla B. Mexican Vanilla C. Tahitian Vanilla  
Fig. 1: Types of vanilla beans



A. Bourbon Vanilla Essence B. Mexican Vanilla Essence C. Tahitian Vanilla Essence  
Fig. 2: Types of vanilla essence

**Curing of Vanilla beans:**

Freshly planted vanilla beans have no flavour or aroma. The curing of harvested vanilla pods are involved four steps-

**Killing:**

Vanilla pods are dipped in hot water (63 to 65°C) for three minutes and retarded the vegetative tissues growth of pods. The enzymatic reaction is initiated and the aroma is developed by killing process. Heating in an oven and/or exposing the bean in direct sun light are few other methods to kill the pods.

**Sweating:**

The pods are wrapped in woollen clothes to maintain the temperature (45–65 °C) of beans and alternate this is stored in air tight wooden boxes during night. This is exposed to sun during day time and continued for nearly 10 days. Thus, the moisture is maintained between 60 and 70% by weight.

**Drying:**

The beans are dried in a wooden rack at room temperature for three to four weeks that results considerable reduction of bean weight up to one third (25 -30%) of the actual. The beans become flexible and stretchy by drying process.

**Conditioning:**

The beans are stored in closed boxes for five to six months and this process is called conditioning. The processed beans are sorted and graded, bundled and wrapped in paraffin paper and preserved till the required quality of bean is achieved, especially flavour and aroma<sup>14</sup>.

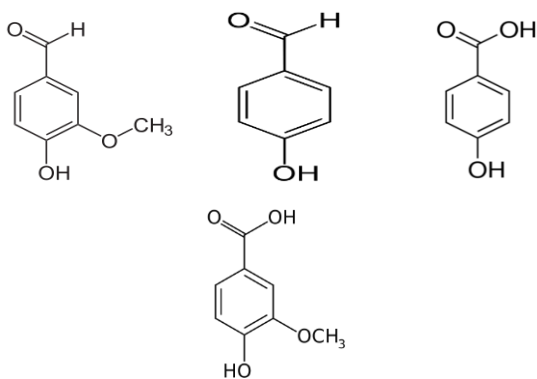
**Constituents and odour of Natural Vanilla:**

The compounds present in vanilla extract determine the aroma. The other non-volatile constituents like tannins, polyphenols, free amino acids and resins which impart the aroma to vanilla<sup>15</sup>.

An extract contains resins retain aromatic compounds for longer. Volatile constituents such as acids, ethers, alcohols, acetals, heterocyclics, phenolics, hydrocarbons, esters and carbonyls are influencing the aroma and flavour of vanilla<sup>16</sup>. Many other compounds- vanillin, p-hydroxybenzaldehyde, guaiacol, and anise alcohol are important for the aroma profile of vanilla. Each vanilla bean only contains around 3% - 5% vanillin by its volume. However, vanillin signifies for about 25% of the total flavour and fragrance experience of genuine vanilla extract and remaining 75% are the organic compounds found in real vanilla beans. This is the major difference between extract and essence. But the bean extract contains three other major components, vanillic acid, 4-hydroxybenzoic acid, and 4-hydroxybenzaldehyde, which account for 17 percent (by weight) of the flavor

chemicals that make up vanilla<sup>17</sup>.

In view of various volatile compounds reported in vanilla extract, vanillin is the single most characteristic component of flavour. Bioactive properties and because of advancements in chemistry and pharmacology, most of the earlier uses of vanilla have given way to functional uses of vanillin, vanilla's main constituent<sup>3</sup> and<sup>18</sup>.



Vanillin 4- hydro benzaldehyde 4-hydroxybenzoic vanillin acid

Fig. 3: Constituents of natural vanilla

#### Canadian regulations:

According to Food and Drug Regulations (C.R.C., c. 870)<sup>19</sup>, vanilla extract products are to be processed/produced from vanilla beans (*Vanilla planifolia* or *Vanilla tahitensis*). In 100 ml of extract, it must have an amount of soluble substances that proportional to their natural state available for extract. Specifically, if the beans contain lesser than 25% water content, the vanilla extract must consist of at least 10 g of vanilla beans; if the beans contain more than 25% water content, the vanilla extract must consist of at least 7.5 g of vanilla beans. Any other colour should not be found in vanilla extract.

#### Culinary Uses of Vanilla:

The most significant flavouring component in many baked items and custards is found vanilla that adds its taste. Vanilla is adding creaminess in sauces, balancing sweetness in desserts, and also adding flavour to tea, toning or masking bitterness and acidity<sup>20</sup>. Vanilla extract is not only delicious in many bakery products and other includes beverages like milkshakes, flavouring drinks and yogurt for a better flavour. Vanilla exhibits antioxidant and antimicrobial activity thus acting as a food preservative<sup>21</sup> and <sup>22</sup>.

#### Health Benefits of Vanilla Extract:

Vanilla bean extract is richer when compare to the artificial and they are not only for their aroma and taste, but these beans in fact have unbelievable health benefits.

- **Helps to treat infection:**

The active compounds present in the plant *Vanilla* such as vanillin and Isoeugenol known to have antifilarial property<sup>23</sup>.

- **Antioxidant activity:**

Antioxidants found in natural vanilla extract are vanillic acid and vanillin, that protects the body from harmful components, such as free radicals and toxins. These antioxidants are used to preserve food and health supplements as nutraceuticals<sup>22</sup>.

- **Antimicrobial activity:**

The active ingredients of the vanilla extract in *Vanilla planifolia* are flavonoid and alkaloid in nature. All the parts of this plant can be a potential source for evolving newer antimicrobial compounds<sup>24</sup>.

- **Anti-inflammatory activity:**

Vanilla extract has anti-inflammatory abilities and preserves liver health<sup>25</sup>.

- **Antinociceptive effect:**

A study conducted by Vanillin is known to have antinociceptive agent<sup>26</sup>.

#### The Problem with Natural Vanilla Production:

Vanilla is a very difficult to cultivate as basically it requires 600 hands for pollination to produce one kg of cured beans. The processing of the beans involves crucial and time consuming to evaluate their aroma and inspect the quality of each bean<sup>27</sup>. Farmer's income is escalating from vanilla by attaining valuable certifications such as organic, fair-trade, and Rainforest Alliance Certification. Perhaps it is difficult to plant more orchids since their area of farms are often quite miniature and the maturation process takes four years for the vines<sup>28</sup>.

Hence food markets face a huge shortage of the vanilla. Food makers, meanwhile, are confronting skyrocketing costs for natural vanilla<sup>29</sup>. To meet the growing demand, food brands have to introduce synthetic vanillin to the market.

#### Synthetic Vanillin:

Synthetic vanillin is an alternate and chemical form for natural vanilla which is made from petrochemicals and by products from the paper industry. This synthetic vanillin is a nature identical vanilla. It is commonly used to reduce production costs. Since it's cheap, available everywhere and vanilla flavoured over-the-counter medicines, beverages, and cookies are found. Thus synthetic vanillin is able to satisfy the demands of the vanillin consumers<sup>2</sup> and <sup>30</sup>.

Both natural and synthetic vanilla contains the same major flavour chemical, vanillin. Natural vanilla has a much richer mouth feel and aroma compared with 'vanilla essence' or 'synthetic vanilla' as it only contains

synthetically derived 'vanillin' hence the lack of diverse flavours<sup>28</sup>.

There are two types of synthetic vanillin- 1. lignin-based and guaiacol based. The lignin-based vanillin is made from wood pulp, has a richer flavour. The guaiacol based vanillin is more cost effective flavour to the shortage of vanilla flavouring<sup>31</sup>.

#### Synthetic Vanillin Contains Chemical Additives:

Synthetic vanillin flavouring is due to its chemical, lignin vanillin which mimics the flavour of natural extract from real vanilla. The first commercial synthesis of vanillin starts with the more readily available natural compound is eugenol. Lignin vanillin is obtained from wastes produced in the paper manufacturing industry. Some vanilla flavouring also contains glycerine or a glycol base<sup>32 and 18</sup>.

#### Ethyl Vanillin:

Ethyl vanillin is also an artificial chemical that tastes like vanilla. Ethyl vanillin, or 3-ethoxy-4-hydroxybenzaldehyde, is 3-4 times as potent as vanillin and can be used to increase the aroma and flavour of an extract. Ethyl vanillin is a chemically synthesized flavouring agent related to vanillin or artificial vanilla. It is three times as strong as artificial vanillin and acts as an imitation vanilla. In the preparation of edible flavour, ethyl vanillin can be used instead of vanillin. Ethyl vanillin appears as a white to light yellow needle crystal or crystalline powder. It has an aroma similar to vanilla beans, but it is more concentrated than vanillin. Ethyl vanillin can be used to flavouring chocolates, candies, biscuits, beverages and ice creams<sup>33</sup>.

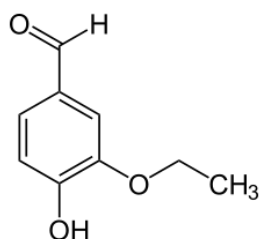


Fig. 4: Ethyl vanillin

#### Health Impact of Synthetic vanillin:

A negligible amount of neurotoxins contained in vanillin are capable of killing brain cells<sup>24</sup>. Ethyl vanillin cause allergic<sup>34</sup> and can irritate the eyes, skin, and the respiratory tract<sup>33</sup>.

#### Acceptable Daily Intake:

The widely industrially produced vanillin is ingested in the form of bakery foods and hot and cold beverages. Remaining is used externally as soaps, perfumes etc. Acceptable Daily Intake (ADI) of vanillin in form of food and beverage is worldwide and it implies that almost every human. An ADI of 10 mg/kg has been

approved by FAO/WHO and EU. For a 70 kg person, the ADI is 700 mg vanillin that corresponds to minimum 700 g chocolate, or 7000 g of ice cream<sup>35</sup>.

#### Food and Drug Administration rules:

Natural vanilla is been a main source for dairy products like ice cream and yogurt for decades. According to Food and Drug Administration rules in the United States, declare that vanilla ice cream must get its flavour from natural vanilla. If it is flavoured partially or through some other source, the company should label "vanilla flavoured" or "artificial vanilla" on the package, a likely turnoff to consumers<sup>36</sup>.

#### Analysis of vanilla Compounds:

- Vanillin is a chief constituent of vanilla extract, a flavouring ingredient which is been used in food products and drinks. Analysis of vanilla Compounds in vanilla extracts and model vanilla ice cream mixes using novel technology has been studied<sup>37</sup>.
- Liquid chromatographic method has been used to quantify coumarin, vanillin, and ethyl vanillin in vanilla extract<sup>38</sup>.
- RPLC method for the characterization of vanilla extract, a key component in food, has been established<sup>39</sup>.
- HPTLC method has been proposed to determine the vanillin in three different food samples such as vanilla essence, custard powder and vanilla flavoured ice cream<sup>40</sup>.

#### CONCLUSION:

Vanilla has a very versatile flavouring agent and is popular worldwide. It is found in all our confectionery products like ice cream, candies, cakes, and cookies. Vanilla also improves perception of sweetness and other flavours. Natural vanilla extract is obtained by curing vanilla beans. Vanilla substitutes are actually nature-identical artificial vanillin (i.e.) synthetic vanillin derivatives synthesized on multi-ton scale from guaiacol or lignin extracts, which can be isolated from wood pulp or petroleum by products. The distinction between natural and artificial vanillin is the source of chemicals. The synthetic chemicals in artificial vanilla flavour generally cost less to produce than finding natural sources of vanilla. Vanillin extracted from nature is safer than artificially made nature identical vanillin. On the whole, artificial vanilla tends to be cheaper, but the health impact is to be considered.

#### REFERENCES:

1. Hoffman AC, Salgado RV, Dresler C, et al. Flavour preferences in youth versus adults: a review. *BMJ*; 2016;0: 1–8. doi:10.1136/tobaccocontrol-2016-053192.
2. Sercarz LL. How vanilla became the world's favorite flavor. *Saveur*, Internet [Updated: February 09, 2018; Accessed: February 12, 2018] [https://en.wikipedia.org/wiki/Vanilla\\_extract#cite\\_ref-1](https://en.wikipedia.org/wiki/Vanilla_extract#cite_ref-1).
3. Sinha AK, Sharma UK, Sharma N. A comprehensive review on

- vanilla flavor: extraction, isolation and quantification of vanillin and others constituents. *Int J Food Sci Nutr*; 2008;59(4): 299-326.
4. Bomgardner M.M. The problem with vanilla. After vowing to go natural, food brands face a shortage of the favoured flavor. *ACS Publications*, 2016; 94(36): 38-42. <https://cen.acs.org/articles/94/i36/problem-vanilla.html>.
  5. Kumar RBK, and TN Balamohan. Factors affecting the quality of Vanilla – A Review. *RRJAAS*, 2013; 2(3): 37-41.
  6. Sharp MD. B.S, Graduate Program in Food Science and Technology, The Ohio State University, Published: July 08, 2009; Accessed: January 17, 2018.
  7. Anandan A. *Vanilla: The Green Gold*. Sura Books. 2004. ISBN: 8174785450.
  8. Balamurugan S. A study of cost and returns of vanilla cultivation in India. Published: January 2009
  9. Sachan D. *Vanilla and its Potential in India*. Exim Bank: Research Brief No. 17 September 2005.
  10. Dr. Mercola. Dr. Mercola's Natural Health Newsletter. Published: December 7 2017.
  11. Charles DJ. *Vanilla*. Published: August 19, 2012; Accessed: March 12, 2018.
  12. Anuradha K, Bellur, N Shyamala and M Naidu. *Vanilla- Its Science of Cultivation, Curing, Chemistry, and Nutraceutical Properties*. *Critical Reviews in Food Science and Nutrition*; 2012; 53 (12): 1250-1276. 1276, DOI: 10.1080/10408398.2011.563879.
  13. Leigh K. *Madagascar Vs. Tahitian Vanilla Beans*. Published: September 28, 2017.
  14. Frenkel DH, JC French, NM Graft, DM Joel, FE Pak, C Frenkel. *Interrelation of Curing and Botany in Vanilla (Vanilla planifolia) Bean*. *Can. Int. Dev. Agency (CIDA)*. 2004: 93- 102.
  15. Gokare R. *Vanilla flavour: Production by conventional and biotechnological routes*. *Journal of the Science of Food and Agriculture*; 2000; 80(3):289 – 304.
  16. Takahashi M, Y Inai, N Myazawa, Y Kurobayashi and A Fujita. *Key Odorants in Cured Madagascar Vanilla Beans (Vanilla planifolia) of Differing Bean Quality*. *Bioscience, Biotechnology, and Biochemistry*; 2013; 77(3): 606-611, DOI: 10.1271/bbb.120842.
  17. T Li and JPN Rosazza. *Biocatalytic Synthesis of Vanillin*. *Applied and Environmental Microbiology*; 2000; 66(2): 684-687.
  18. Kumar R, P. K. Sharma, PS Mishra. A Review on the Vanillin derivatives showing various Biological activities. *International Journal of PharmTech Research*; 2012; 4(1): 266-279.
  19. *Food and Drug Regulations: (C.R.C., c. 870), Flavouring Preparations*. Branch, Legislative Services. "Consolidated federal laws of Canada, Food and Drug Regulations". [laws-lois.justice.gc.ca](http://laws-lois.justice.gc.ca). Accessed: January 09. 2018.
  20. Cadena R.S, A. G. Cruz, J. A. F. Faria, and H. M. A. Bolini. *Reduced Fat and Sugar Vanilla ice creams: Sensory profiling and external preference mapping*. *Journal of dairy Science*; 2012; 95(9): 4842-4850. DOI: <https://doi.org/10.3168/jds.2012-5526>.
  21. Jamal Uddin A.F.M, A. Nusrat, S. Parvin, M.Z.K. Roni and U. Mayda. *Antibacterial and Antifungal Activities of Vanilla Planifolia Grown in Sher-E-Bangla Agricultural University*. *Bangladesh Research Publications Journal*; 2015;11(1): 34-39.
  22. Shyamala B.N, M. Madhava Naidu, G. Sulochanamma, and P. Srinivas. *Studies on the Antioxidant Activities of Natural Vanilla Extract and Its Constituent Compounds through in Vitro Models*. *J. Agric. Food Chem*; 2007;55 (19): 7738–7743. DOI: 10.1021/jf071349.
  23. Ndjonka D, Rapado LN, Silber AM, Liebau E, Wrenger C. *Natural Products as a Source for Treating Neglected Parasitic Diseases*. *International Journal of Molecular Sciences*; 2013;14(2):3395-3439. doi:10.3390/ijms14023395.
  24. Shanmugavalli N, V. Umashankar and Raheem. *Anitmicrobial activity of Vanilla planifolia*. *Indian Journal of Science and Technology*; 2009;2(3): 37-40.
  25. Makni M, Chtourou Y, Fetoui H, Garoui el M, Boudawara T, Zeghal N. *Evaluation of the antioxidant, anti-inflammatory and hepatoprotective properties of vanillin in carbon tetrachloride-treated rats*. *Eur J Pharmacol*; 2011; 668(1-2):133-9.. doi: 10.1016/j.ejphar.2011.07.001.
  26. Niazi J, Kaur N, Sachdeva RK, Bansal Y, Gupta V. *Anti-inflammatory and antinociceptive activity of vanillin*. *Drug Dev Ther*; 2014;5: 145-7.
  27. Pokorná I, L. Smutka. *Is there any future for cash crops in developing countries? The case of vanilla*. *Agris on-line Papers in Economics and Informatics*; 2011; III (1): 23-31. Available: [http://ageconsearch.umn.edu/bitstream/102491/2/agris\\_online\\_2011\\_1\\_1\\_pokorna\\_smutka.pdf](http://ageconsearch.umn.edu/bitstream/102491/2/agris_online_2011_1_1_pokorna_smutka.pdf). Accessed: April 21, 2018.
  28. Gallage NJ, BLMøller. *Vanillin–Bioconversion and Bioengineering of the Most Popular Plant Flavor and Its De Novo Biosynthesis in the Vanilla Orchid*. *Cell Press*; 2015; 8(5): 40-57.
  29. Howard R.L, Abotsi E, Jansen van Rensburg E.L. and Howard S. *Lignocellulose biotechnology: issues of bioconversion and enzyme production*. *African Journal of Biotechnology*; 2003;2(12): 602-619.
  30. C. Rose Kennedy. *The Flavor Rundown: Natural vs. Artificial Flavors*. SITN. Updated: September 21, 2015; Accessed: February 10, 2018. Available: <http://sitn.hms.harvard.edu/flash/2015/the-flavor-rundown-natural-vs-artificial-flavors/>
  31. Dr. B Maes. *Is Vanillin Bad For You?* Internet [2018 March 05] Available: <https://www.isitbadforyou.com/questions/is-vanillin-bad-for-you>.
  32. Wolf N. *Advantages and Disadvantages of Imitation Vanilla Flavoring*. *Livestrong.com*, Published: October 3 2017; [ January 10, 2018]. Available: <https://www.livestrong.com/article/436468-advantages-and-disadvantages-of-imitation-vanilla-flavoring/>
  33. Sky Z. *Ethyl vanillin – toxicity, side effects, diseases and environmental impacts*. *Chemical News*. Updated: December 05, 2017. Internet [ January 03, 2018] Available: <http://www.chemicals.news/2017-12-05-ethyl-vanillin-toxicity-side-effects-diseases-and-environmental-impacts.html>.
  34. Andrew. *Natural and Artificial Flavors*. Internet [Updated: October 28, 2010. Accessed: February 21, 2018] Available: <https://eatingrules.com/natural-flavors-artificial-flavors/>
  35. *UNEP Publications, Vanillin, OECD SIDS*.
  36. *Pacificgourmet. Vanilla Wavers*. Internet [Updated on: December 8, 2016] Available from: [http:// www.pacgourmet.com/vanilla-wavers/](http://www.pacgourmet.com/vanilla-wavers/).
  37. Sujalmi S, Suharso, R. Supriyanto and Buchari B. *Determination of Vanillin in Vanilla (Vanilla Planifolia Andrews) from Lampung Indonesia by High Performance Liquid Chromatography*. *Indo. J. Chem*; 2005; 5 (1): 7 – 10.
  38. Jain P.K and Himanshu Josh. *Coumarin: Chemical and Pharmacological Profile*. *Journal of Applied Pharmaceutical Science*; 02 (06); 2012: 236-240.
  39. Lavine BK, DT Corona, UNDT Perera. *Analysis of vanilla extract by reversed phase liquid chromatography using water rich mobile phases*. *Micro Chemical journal*; 2012; 103:49-61.
  40. Veni K, Meyyanathan S N, AR Aduri, SS Alkeshbhai, Elango K. *Analysis of Vanillin in Food products by High Performance Thin Layer Chromatography*. *J Adv Sci Res*; 2013, 4(1): 48-51.