Vanilla beans, both green and cured were obtained from one of the farmers located in the chickmangalore distract of Karnataka, India during 2006-07 and immediately stored in frozen condition until use. Seeds were collected from the beans by opening the fruit along the suture lines and seeds were scraped and stirred in distilled water using magnetic stirrer to remove adhering tissues. The procedure was repeated several times until the seeds were devoid of any placental tissues and other debris. Seeds were dried on tissue paper in the laboratory at room temperature and used for further studies.

(i) Anatomical studies : As mentioned above
(ii) Moisture determination : As mentioned above
(iii) Solvent extraction

Briefly, 10 grams of seed was ground using liquid nitrogen with pestle and mortar. Fine powder thus obtained was extracted using the solvent methanol and hexane (50 ml each) for the extraction of polar and non polar seed constituents respectively. The slurry was filtered using whatman No.1 filter paper and again extracted twice with 50 ml of methanol and hexane respectively. Further the filtrate was concentrated under reduced pressure and made into a percentage solution (1%).

(a) Spectra of Methanol extract

Small portion of methanol extract was subjected to UV Visible (190 to 700 nm) spectrum scanning to check any changes in the absorption using HELIOSa, 82220, v 4.2 spectrophotometer.

(b) TLC of extracts

Exactly 0.03 ml of the samples was loaded onto a analytical TLC plate (TLC Silica gel 60 F254, Merck, Germany) and developed using hexane:diethyl ether and acetic acid (80:20:1) as mobile phase for hexane extract, whereas methanol extract was developed in a toluene:ethyl formate:formic acid (50:40:10) as mobile phase. Bands were visualized by spraying with 10% sulphuric acid and heating the plate at 125°C for about 1.5 hours and observed for the banding pattern and photographed.

(iii) Estimation of vanillin and other phenolics by HPLC: As mentioned above