SUMMARY AND CONCLUSIONS
Colorectal adenocarcinomas account for the third leading cause of cancer deaths. The incidences are on the rise globally, especially in the Asian countries. There is no correlation seen between genetic predisposition and the incidences recorded which implies that non-hereditary causes of colorectal cancers seem to be responsible for the large numbers of people suffering from this cancer.

Natural products offer a means for the discovery of novel chemical moieties with specific reactivity. If explored systematically, new chemical moieties from plants can be isolated and may be used for the treatment of such disorders. This is especially more significant in cancer treatment since cancer cells are known to develop drug resistance quickly.

This work involved screening of some plants for anticancer activity against colorectal adenocarcinomas. Ten plants of seven families were selected and their extracts screened for anticancer activity in vitro using the MTT assay. Two adenocarcinoma cell lines viz Colo205 and HT-29 were chosen for the study. The main results of screening were as follows:

1. Four extracts of three plants were found to have promising activity in vitro. These were the petroleum ether and dichloromethane extract of *Costus speciosus*, the dichloromethane extract of *Vanda tessellata* and the dichloromethane extract of *Vernonia cinerea*.

2. These extracts were further screened for their cytotoxic potential on INT407, the intestinal cell line of embryonic origin. Of the four extracts, it was seen that the dichloromethane extract of the aerial parts of the plant *Vanda tessellata* was found to possess selective toxicity on the adenocarcinoma cell lines, whilst exhibiting minimal toxicity on the normal intestinal cells.

The active extract was also compared for its cytotoxic potential against 5-fluorouracil (5-FU) -the first line chemotherapeutic agent used in colorectal cancers. It was found that the extract was equipotent on the adenocarcinoma cells as 5-FU in vitro; however, while 5-FU was equally toxic to the normal cells, the active extract exhibited minimal toxicity on the same.
The dichloromethane extract of *Vanda tessellata* was further investigated for its mechanism of anticancer activity. It was found that:

1. It drastically decreased mitochondrial membrane potential in the adenocarcinoma cell lines.
2. It did not cause any drastic increase in the reactive oxygen species levels at the IC$_{50}$ concentration.
3. It caused an arrest in the cell cycle in the G0/G1 phase of cell growth in both the adenocarcinoma cell lines.
4. It caused death via the apoptotic pathway (as seen by the appearance of DNA ladder- the hallmark of apoptotic death).

The clonogenic assay revealed that the effect of exposure to the dichloromethane extract caused an irreversible damage to the cells since the treated cells were incapable of forming as many colonies as the untreated cells under identical conditions.

The dichloromethane extract was then subjected to qualitative phytochemical analysis to identify the phytoconstituents. It was found to contain steroidal molecule/s and glycoside/s.

Various fractions from the active extract were isolated using the HPTLC technique. They were screened for bioactivity. Some fractions were found to cause proliferation while some fractions were cytotoxic to the adenocarcinoma cell lines. The cytotoxic fractions were further evaluated for their toxicity potential on INT407. It was observed that some of the isolated fractions were almost equitoxic to both, the normal intestinal cells as well as on colorectal adenocarcinoma cells (as evident from the similar IC$_{50}$ concentrations on the three cell lines). This was in contrast to the selective toxicity seen in the whole extract. Also, the IC$_{50}$ concentrations of the individual fractions were similar to or greater than the IC$_{50}$ concentration of the DCM extract on Colo205 and HT-29. This may suggest that the selective cytotoxicity of the extract towards the adenocarcinoma cells could be attributed to synergistic action of more than one component present in the same.
Two of the isolated fractions were further investigated for their effect on the cell growth cycle and the ability to cause apoptosis. Fraction 3 was more capable of causing an arrest in the G0/G1 phase in all the three cell lines. Fraction 6 was found to cause a greater cell cycle arrest in HT-29 as compared to Colo205. Fraction 3 was more capable of causing apoptosis than fraction 6. Both the fractions did not cause much apoptosis in INT407. Thus fraction 3 seemed to be capable of causing both, cell cycle arrest as well as apoptosis, while fraction 6 caused better cell cycle arrest than apoptosis.

Characterization studies on these fractions revealed the presence of two different steroidal molecules in the two fractions. However, HPLC analysis also showed presence of some impurities in both the fractions. $^1$H, $^{13}$C NMR and IR spectroscopic studies probably indicate the presence of two steroidal molecules: melianin (in fraction 3) and a withadienolide (in fraction 6) which have been previously reported to be present in this plant. Further purification work will be required to confirm the same.

Thus, the anticancer activity of the plant *Vanda tessellata* which is being reported here for the first time, may be attributed to the presence of melianin and the withadienolide in the dichloromethane extract of the aerial parts of the plant.