

4. Discussion, conclusions and recommendations

4.1. Discussion:

Since the late 1960s plant lectins were recognized as readily available proteins which are invaluable tools for the study of carbohydrates in a solution and on a cell surface, and also the findings that some plant lectins (such as wheat germ) agglutinated malignantly transformed cells but not their normal parental cells, and the reports provided compelling evidence that cancer might be associated with a change in cell- surface sugars. Other striking reports linked between some plant lectins and their capabilities to inhibit the adherence of some pathogenic microorganisms to cells (Sharon 2008).

Several proteins of plant origin are powerful inhibitors of protein synthesis, and can be divided into two categories. The first includes three highly toxic lectins, ricin, abrin and modeccin, which inhibit protein synthesis in intact cells and in cell-free systems (Olsnes et al. 1974). The second category includes several proteins scarcely toxic to animals, which inhibit protein synthesis in cell-free systems, but have little or no effect on whole cells. These are a *Phytolacca americana* peptide, crotins and curcins, a protein from wheat germ, and a number of unidentified proteins from seeds. It is well established that the toxic inhibitors act by inactivating irreversibly and in a catalytic manner (i.e. enzymically) the 60s ribosomal subunit, which becomes unable to bind elongation factor 2. The non-toxic proteins when purified or semipurified act in the same way, except for the inhibitor from wheat germ, whose action is ATP-dependent (Barbieri et al. 1979).

Momordica charantia is a close relative of *M. balsamina*, which has been extensively studied. Lin et al. (1978) purified two lectins from the seeds of *Momordica charantia* (bitter pear melon, a cucurbit): one of these, called

Momordica charantia agglutinin, was a potent haemagglutinin, whereas the other one, called momordin, had less haemagglutinating power and was a moderate inhibitor of growth of cancer cell lines (Barbieri et al. 1979).

Momordica balsamina also known as 'Balsam apple' (African pumpkin), is an important medicinal and nutritional plant of Cucurbitaceae. It is an annual to perennial tendril-bearing herb native to tropical regions of Africa. In India, it occurs naturally in forests, in the rainy season. The leaves, fruits, seeds, and bark are reported to have various medicinal and nutritional importance and are called 'Hidden gift of Nature (Thakur et al. 2009). Momordin isolated from *Momordica balsamina* is capable of inhibiting the growth of HIV and other viruses (Ortigao & Better 1992). The leaves and fruit extracts of this plant show antiplasmodial activity and are being used against malaria in African traditional medicine. The extracts of various parts of this plant show shigellocidal, anti-diarrheal, antiseptic, antibacterial, antiviral, anti-inflammatory, hypoglycemic and antimicrobial properties (Thakur et al. 2011).

In the present study, we attempt to purify and characterize *Momordica balsamina* seed lectin and to study its modulating effects on a group of commercial cancer cell lines. The first step to achieve the purification of MbSL was by the detection of the lectin by hemagglutination test (the activity test of lectins). The crude protein extracts of *Momordica Balsamina* Seeds revealed high hemagglutinating activity when tested against human and animals RBCs and showed more activity towards the O blood type (as shown in Table 3.1) which could be inhibited by many sugars (as shown in Table 3.2). These results agreed with results of *Momordica charantia* agglutinin which was found to be able to agglutinate the human O type red blood cells and the agglutination was inhibited by galactose or its derivatives (Lin et al. 1978).

The first step in the purification of a lectin is to decide the best type of affinity chromatography matrix that should be used. This requires determining the lectin sugar specificity. It is known from other researchers in Cucurbitaceae family lectins that they tend to be galactose specific as was found for lectins from the closely related species *Momordica charantia* (Huang et al. 2008) and lectin from the seeds of *Trichosnthes dioica* (Sultan et al. 2004). Since lactose was a stronger inhibitor of hemagglutination than galactose, the purification was done in a single step by affinity chromatography on alpha agarose lactose matrix.

The molecular weight of the *Momordica balsamina* seed lectin (MbSL) was examined by gel filtration of the crude protein extract on sephadex G-100. The native molecular weight of MbSL, as determined by gel filtration, is 81 kDa (as shown in Figure 3.5). The purified lectin was further analyzed by reducing SDS-PAGE (with β -mercaptoethanol) for revealing its subunit molecular mass and the result showed a single sharp band around 30 kDa (as shown in Figure 3.6). These results suggested that, the lectin is a homodimer and the difference in molecular weight due to other proteins, which is probably RIP subunit of the protein. This is similar to the results obtained for another lectin, Ebulin 1, purified from the plant *Sambucus ebulus* L leaves ,which is composed of two subunits, a catalytic A subunit of 26 kDa and a D-galactose-binding lectin B subunit of 30 kDa (Tomas et al. 1993). In comparison, the native molecular mass of *Erythrina speciosa* seeds lectin when detected by hydrodynamic light scattering was 58 kDa and when examined by mass spectroscopy and SDS-PAGE it was found to be composed of two identical subunits of molecular mass of 27 kDa (Konozy et al. 2002).

Examination of the effect of pH on the activity of purified MbSL was conducted in a pH range, from 2-13. Results showed that the activity of the lectin remained stable in the pH range 2-12 (Figure 3.7). In comparison, *Trichosnthes dioica* lectin

remains stable in the pH range 6-12 was only partially stable at pH 4, while 60% activity was lost at pH 2 (Dharkar et al. 2006). *Erythrina speciosa* seeds lectin was acidic pH sensitive and lost its activity when incubated with all pH values between pH 3 and pH 6. In the pH range 6 to 9.6 there was no effect on the lectin activity (Konozy et al. 2003).

The effect of thermal denaturation on purified MbSL was tested in the temperature range 20-90 °C for 30 minutes. Results showed that the lectin hemagglutinating activity remained stable below 50 °C. Above 50 °C lectin hemagglutinating activity was gradually lost and it was totally inactivated at 90 °C (Figure 3.8). These results revealed the high thermal stability of this lectin as *Erythrina speciosa* seeds lectin which remained significantly stable below 65 °C for more than 90 minutes without losing its hemagglutinating activity. Above 65 °C lectin activity was gradually lost and was totally inactivated at 80 °C, when incubated for less than 10 minutes (Konozy et al. 2003).

Purified MbSL activity slightly decreased against denaturation with different concentrations of urea ranged from 1M – 8M, with significant drop at 3M (Figure 3.9). The stability of any protein varies according to changes in different conditions like pH, temperature and denaturation agents. The three dimensional structure of a protein is held together by non-covalent interactions viz. hydrogen bonds, ionic interactions, hydrophobic interactions, van der Waals' forces and covalently by disulfide linkages. Conditions, which disturb these stabilizing forces in a protein, affect the native conformation of the protein by changing its physical properties and biological activity (Abdul et al. 2002).

For N-terminal sequencing, the purified *Momordica Balsamina* seeds lectin was further purified by reversed-phase fast protein liquid chromatography (Figure

3.10). The eluted peaks 1 and 2 were sequenced by Edman degradation (Figure 3.11) and showed the same N-terminal sequence for the first 15 amino acid residues. It is probable that peak 1 and 2 are isoforms of MbSL. However, once they are eluted in two different peaks, they probably exhibit some modifications in other regions from the complete primary sequence, e. g. different amino acid residues or degrees of oxidation of some amino acid residues.

A BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (by using peak 1, the highest peak) for MbSL in the protein sequence database found significant degree of identity to *Momordica charantia* lectin 1 (MCL1) (Figure 3.12). As a result of sequence comparisons with other lectins, MbSL seemed to be another type of ribosome inactivated protein type II (RIP II) as published by Marcelo Ortigao and Marc Better (Ortigao & Better 1992). However, Ortigao and Better did not attempt to characterize the protein as lectin.

In vitro evaluation of cytotoxicity of purified lectin was carried on four types of commercial cancer cell lines, using the MTT assay. The MTT assay depends on the conversion of tetrazolium salts to insoluble formazan which has a purple color measured at 550 nm.

The four cell lines tested were: AGS (Human Gastric Adenocarcinoma), MKN45 (Human Gastric Cancer), U87-MG (Human Glioblastoma) and ECV-304 (Human Urinary Bladder Carcinoma).

MbSL did not cause any cytotoxic effects on any of the tested cancer cell lines, as indicated by the MTT assay (figures 3.13, 3.14). These results agreed with the results of Ebulin1, a nontoxic ribosome-inactivating protein type 2 from *Sambucus ebulus* leaves which was not toxic to mice or to cultured mammalian cancer cells (Tomas et al. 1993). This allowed us to classify *Momordica balsamina* seed lectin

as a non-toxic RIP type 2, like Ebulin 1, unlike ricin, abrin, viscumin, modeccin, and volkensin, which are toxic RIP type 2.

In contrast to *Momordica balsamina*, *Momordica Charantia* related products were reported by several investigators to have effects on a number of cancer cell lines, including cell cycle arrest and apoptosis without affecting normal cell growth(Fang et al. 2012).

4.2. Conclusion:

In the present study, a lactose-binding lectin was isolated and partially characterized from seeds of *Momordica balsamina* medicinal plant. MbSL shares a high degree of similarity with other Cucurbitaceae family lectins in term of their physicochemical features including sugar specificity, effect of pH and temperature and urea on lectin stability. The results of molecular weight examination (native and subunit) suggested that the protein is a homodimer with two subunits, a lectin and RIP. After N-terminal sequencing of the lectin by using Edman degradation, the search for identity of the lectin by using BLAST in protein sequence database was done, and found significant degree of identity to RIP type 2 of *Momordica charantia* lectin. Although MbSL was found to be one of RIP 2, it showed no effect on the growth of four different human commercial cancer cell lines. This result identified MbSL as non toxic type 2 RIP.

4.3. Recommendations

- 1.** Further studies are recommended in order to achieve complete identification of MbSL as a protein, by doing further extensive characterization of MbSL including studying of secondary and tertiary structure and effect of metal ions on lectin activity.
- 2.** Although MbSL showed no effect on the group of commercial cancer cell lines used in the study, I recommend studying the possible modulating effects of the MbSL on other types of commercial cancer cell lines than tested here.
- 3.** I recommend also studying the effect of MbSL on pathogenic bacteria, pathogenic fungus, pathogenic parasites as well as pathogenic viruses to help in using of the lectin as therapeutic agent.