1. Introduction and literature review

1.1. Introduction

Lectins, multivalent cell-agglutinating proteins, by virtue of their exquisite sugar specificities are useful tools in widespread applications for monitoring the expression of cell-surface carbohydrates as well as for the purification and characterization of glycoconjugates (Kestwal et al. 2007). Accordingly, lectin can be described as a substance which can agglutinate cells or precipitate glycoconjugates, with a structure resembling a carbohydrate binding protein or glycoprotein and is not of immune origin (Vasconcelos et al. 2009).

Lectins have been known since the turn of the 19th century. However, for a long time they attracted little attention, especially as it was assumed that they were confined to the plant kingdom and not present in humans or other animals (Esko & Sharon 2009). It is thought that lectins play an important physiological role as recognition molecules within a cell, between cells, or between organisms (Lin et al. 2010). Lectins are involved in the control of many biological systems.

Plant lectins serve as defense mechanism against insects and fungi. Legume lectins mediate the interaction between the bacteria that fix nitrogen and legume roots in symbiosis (Sharon 2007; Chan et al. 2013). Lectins, such as galectins and collectins, have the ability to act as cytokines, chemokines and growth factors (Croci et al. 2013).

Lectins located in the intracellular compartments serve in trafficking, sorting and targeting of glycoproteins, protein folding, fertilization and development. Lectins function in intracellular translocation of glycoproteins (Dommett et al. 2013; Souza et al. 2013).
A multitude of plants have been identified and used for the treatment of different diseases throughout the world, especially in poor countries. Much research has been focused on the scientific evaluation of traditional tropical plants as drugs; *Momordica charantia* (belongs to Cucurbitaceae family, genus: *Momordica*) has been commonly or frequently used (in traditional medicine) as an anti-cancer agent and anti-diabetic agent and it is often described as food of medicine (Huang et al. 2008).

Many plants produce ribosome inactivating proteins (RIP) which are potent inhibitors of eukaryotic protein synthesis. Ribosome inactivating proteins are classified to RIPs I and RIPs II.

RIPs hydrolytically cleave the N-glycosidic bond of a specific adenine in a highly conserved region of the 28s rRNA. Plants of the genus *Momordica* produce a number of related Type I ribosome inactivating proteins known as momordins or momorcharins. The gene encoding one member of this family, momordinI has previously been cloned, and the N-terminal protein sequences of three Momordica RIPs have been described. Momordins are homologous to other plant RIPs, including the trichosanthins, a multigene family of RIPs produced by the related plant *Trichosanthis kirilowii* (Ortigao & Better 1992).

The use of plants in traditional medicine is now attracting the attention of many scientists to examine the active components and to study toxicity for more safe valuable practice. Many of traditional medicinal plants have been studied for their protein content and lectin activity. However, only about 1% of these are proved through scientific studies to have real therapeutic value when used by humans (Bhaskar et al. 2012).

The present investigation has been devoted to purify, characterize and evaluate the cytotoxicity of lectin from seeds of *Momordica balsamina*, a climber belongs to the family Cucurbitaceae, known locally as African pumpkin, and balsam fruit or
apple in English. This species is closely related to *Momordica charantia* (Bitter melon) which is found in rich savannah (Kaur et al. 2012a; Kaur et al. 2012b).

1.2. Literature review

1.2.1. Lectins

Lectin, a word of Latin origin, is a substance which is not triggered by antigenic stimulations in the immune system and which combines with an antigen in a way resembling that of an antibody. Lectins are multivalent proteins or glycoproteins of non-immune origin that reversibly and non-enzymatically bind carbohydrates with high specificity for the chemical structure of the glycan array without changing their structure (Sharon & Lis 2004a).

Peumans and Van Damme (1995) extended the definition of lectins to include any protein that has non-catalytically carbohydrate binding domain (De Hoff et al. 2009).

Lectins have been mostly isolated from plant seeds although recently have been known to be found in other plant tissues, prokaryotes and higher animals (Lam et al. 2009). However, for a long time they attracted little attention, especially as it was assumed that they were confined to the plant kingdom and not present in humans or other animals (Amna Elhag. 2010). The attitude towards plant lectins began to change in the late 1960s with the realization that these readily available proteins are invaluable tools for the study of carbohydrates both simple and complex, in solution and on cell surfaces, as well as for cell characterization. Much excitement was created by the findings that lectins, such as those of wheat germ (Nagata & Burger 1972), jack bean (*Canavaliaen siformis*, concanavalin A) (Marikovsky et al. 1974), and soybean (Lis et al. 1970) agglutinated malignantly transformed cells but not their normal parental cells. The reports provided compelling evidences that cancer might be associated with a change in cell-surface
sugars, this led to the application of lectins to cancer research. Among the scientists who, at the time, became fascinated by the findings about the selective action of lectins on malignant cells was James D. Watson who said: “can’t we cure cancer by adding those cell agglutinating glycoproteins that specifically attach to the surface of the cancer cell and generate a signal which stops DNA replication? Perhaps someday we may be able to find compounds which would not generate an immunological response (as the lectins will) and yet somehow would specifically cover up the crucial portion of the cancer cell” (Sharon 2008).

Several fundamental features of membranes were revealed, or their existence confirmed, by lectins. They included the demonstration that the oligosaccharides of the plasma membrane of eukaryotic cells are asymmetrically distributed and confined to the outer surface of the cell (Nicolson 1974). Experiments with lectins showed that the oligosaccharides could move in the plane of the membrane, thus providing support to the fluid mosaic model of membrane structure, according to which the membrane consists of proteins and glycoproteins floating in a lipid bi-layer. Lectins were originally obtained by classical biochemical techniques. The introduction in 1965 of affinity chromatography on immobilized carbohydrates for plant lectin isolation greatly facilitated the task and led to a fast increase in the number of purified proteins (Agrawal & Goldstein 1965). Another important discovery made somewhat later was that lectins can be employed for the isolation of glycoproteins (Donnelly & Goldstein 1970); subsequently it was found that they can also be used for the separation of glycoproteins into their glycoforms (Kaneko et al. 2006).

The finding in 1975, that immature thymocytes could be separated from the mature cells by selective agglutination with PNA (peanut agglutinin) was the first demonstration that lectins could be employed for fractionation of cells into biologically distinct subpopulations (Reisner, Linker-Israeli, & Sharon 1976).
introduced the use of PNA as a marker for thymocyte maturation (Daniels, Hogquist, & Jameson 2002), and was followed by the demonstration that mouse bone marrow and spleen cells fractionated by PNA and SBA (soya bean agglutinin) could be utilized for bone research (Reisner et al. 1978). This paved the way for the application of SBA for the purging of human bone marrow from haploidentical donors for transplantation into children born with SCID (severe combined immune deficiency or ‘bubble children’) (Reisner et al. 1983). By now over 75% of those hundreds of bubble children who received transplants of SBA-purged bone marrow have been cured and lead a normal life. Numerous additional widely used direct and indirect applications of plant lectins, either in their native form or as soluble and immobilized derivatives, were subsequently developed (Sharon & Lis 2004a).

Of special significance are the lectin resistant cells that permitted the deciphering of several pathways of protein glycosylation and were also adopted by the biotechnology industry for the production of glycoprotein drugs (Patnaik & Stanley 2006).

A very recent advance is the introduction of plant lectins in the form of microarrays as a unique means for high throughput analysis of protein glycosylation (Rosenfeld et al. 2007) and for profiling global changes in mammalian and bacterial cell surface glycomes (Tateno et al. 2007).

By 1973, nearly a dozen of lectins had been characterized, the amino acid composition of only a few of these was known and the three-dimensional structure of just a single lectin, concanavalin A, had been solved (Amna Elhag. 2010).

Lectins have many biological properties in common, they represent a diversified group of proteins with respect to size, composition, and structure a prediction fully confirmed by the hundreds of lectin sequences and structures that have now been elucidated (Lis & Sharon 1973).
No wonder that a leading glycobiology textbook singled out plant lectins as the proteins that “have helped to catapult the field of glycobiology into the modern era” and have made “an enormous contribution to modern biochemistry. The lessons learned about plant lectin isolation, characterization and assays of their binding activity, directly contributed to modern breakthroughs in the discovery of the C-type and S-type lectins” (Crocker et al. 1998).

In the 1980s, the combining site of the *E. coli* type-1 fimbriae was mapped, mainly by measurement of the ability of a variety of α-mannosides and mannose-containing oligosaccharides to inhibit the agglutination of yeasts by the bacteria or by the isolated fimbriae (Firon et al. 1983). Hydrophobic mannosides in particular proved to be powerful inhibitors of the agglutination reaction, up to three orders of magnitude better than methyl α-mannoside (Firon et al. 1987).

The studies also proved that blocking of bacterial lectins might prevent infection in vivo. Infection of mouse bladder with a mannose-specific *E. coli* strain was markedly diminished by pre-suspension of the bacteria in a solution of methyl α-mannoside but was not affected by glucose, a sugar to which the bacteria do not bind (Aronson et al. 1979; Bouckaert et al. 2005).

The prophylactic effects of adhesion-inhibitory saccharides have been demonstrated in several other animal models, such as pneumococcal pneumonia in rats and *Helicobacter pylori* gastric infection in monkeys (Sharon 2006).

In addition to demonstrating unequivocally that recognition of cell surface carbohydrates is a prerequisite (Sharon & Lis 2004a) for infection by lectin-carrying bacteria, that data serve as a definitive proof for the validity of the concept of antiadhesion therapy of microbial diseases (Wellens et al. 2008). However, success of such treatment in humans has not yet been achieved. In addition to their role in initiation of infection, mannose specific bacterial surface
lectins may also mediate the attachment of the bacteria to phagocytic cells in the absence of opsonins, and be taken up and eliminated by these cells, a phenomenon named lectino-phagocytosis (Ofek & Sharon 1988).

Additionally, the bacteria and yeasts that are coated with mannosides can bind to mannose-specific lectins present for example on macrophages [such as the MMR (macrophage mannose receptor)] and be similarly eliminated by lectinophagocytosis. This was an early example of innate immunity that acts before the induction of an antibody-mediated response, in which lectins are major players. Among the lectins of pathogenic protozoa, the best characterized are those of *Entamoeba histolytica*. One of these, specific for *N*-acetylgalactosamine, mediates the adhesion of the amoebae to host cells and determines the severity of infection (Frederick et al. 2005).

### 1.2.1.1. Plant lectins mode of action

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. 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. enzymatically) the 60S ribosomal subunit, which becomes unable to bind elongation factor 2. The non toxic proteins when purified or semi purified act in the same way, except for the inhibitor from wheat germ, whose action is ATP-dependent (Barbieri et al. 1980).
1.2.1.2. History of lectins

In 1888 Peter Hermann Stillmark presented the earliest description of hemagglutinins. Stillmark isolated a protein from the seeds of castor tree (*Ricinus communis*) and he named it ricin which was highly toxic. Boyd and Reguera, 1949 and Renkonen, 1948 investigated saline extracts of hundreds of plants for hemagglutination activity, they demonstrated that some plant hemagglutinins are blood type specific. On this basis Boyd gave the term lectins to describe those blood type specific plant hemagglutinins (Sharon 2008).

In 1952, Watkins and Morgan verified the inhibitory effects of sugars on lectin activity; since that time terms such as D-mannose binding lectin and D-galactose binding lectin have been denoted to describe lectin sugar specificity (Ng et al. 1989). The first lectin to be crystallized was concanavalin A (Con A) (*Canavalia ensiformis*) by James B. Sumner on 1919; but only on 1936 Sumner reported its agglutination behavior towards cells such as erythrocytes and yeast (Amna Elhag. 2010).

Con A is the first lectin to be known to agglutinate tumor cells; in addition its primary and three dimensional structures are the first to be revealed among all lectins (Sharon 2007). Lectins have been isolated from animals, bacteria, fungi and viruses (Sharon 2007; Wang & Ng 2001).

There are several lectin families which vary in its structure, specificity and biological activity. Plant lectins are the most studied lectins, whereas legume lectins are the most studied plant lectins.

1.2.1.3. Classification of lectins

Lectins classification is a challenge because of their diversity. It is still evolving area and general agreement has not been achieved yet.
Arason (1996) proposed a classification that includes six families according to the carbohydrate binding domain: Legume lectins, Cereal lectins, P-type lectins, S-type lectins, C-type lectins and Pentraxins. The first two are plant lectins while the other four are from animal origins (De Hoff et al. 2009).

Lectins are furthermore divided according to their carbohydrate-binding domain into: Merolectins which have a single carbohydrate-binding domain; found on certain microorganisms (Actinomyces, Myxococcus and Mycoplasm among others) and are incapable of agglutinating cells. Hololectins contain at least two identical or very homologous carbohydrate-binding domains; Hololectins are true agglutinins. Chimerolectins consist of one or more carbohydrate-binding domains which are attached to another unrelated domain with a separate biological function. Superlectins contain at least two carbohydrate-binding domains (Liu et al. 2009). In addition lectins have been classified according to their sugar-binding specificity to monospecific and polyspecific, the later can interact with more than one sugar (Neutsch et al. 2012).

Recent studies that incorporate sequence homology, structural relatedness showed that classical classification that depended on carbohydrate specificity could be unreliable because lectins, as determined by glycoarray analysis, generally show higher binding affinities to complex oligosaccharides than to simple monosaccharides. Given this classical lectin classification is supplanted by recent more informative homology-based systems; such as the recent 2008 classification of Van Damme et al. (De Hoff et al. 2009; Sharon & Lis 2004b).

Van Damme et al. (2004) classified plant lectins on the base of their carbohydrate-recognition domain (CRD) into seven families: amaranthins, Cucurbitaceae phloem lectins, lectins with hevein domain(s), jacalin related lectins, legume
lectins, mannose-binding lectins from monocots, and type II ribosome-inactivating proteins (RIPs). On 2008 Van Damme et al. updated their CRD-based classification from seven to twelve families (De Hoff et al. 2009). This classification incorporated sequence and structural homology as well as evolutionary relatedness (De Hoff et al. 2009).

**Ribosome inactivating proteins**

RIPs (ribosome-inactivating proteins) are potent toxins that kill cells by inactivating ribosomes (Casellas et al. 1988).

RIPs catalytically depurinate major rRNA by exerting a highly specific N-glycosidase activity that cleaves an adenine base, A4324, which forms part of a tetranucleotide ‘GA4324GA’ sequence in a conserved loop in rat28srRNA termed the SRL (sarcin-ricin loop) (Azzi et al. 2009).

The removal of this adenine causes structural changes in the rRNA that disrupt binding of elongation factors to the ribosomes, and as a result protein synthesis is arrested at the translocation step (Barbieri et al. 2004).

RIPs have been divided into two categories: the type I RIPs such as saporin, pokeweed antiviral protein and tricosanthin, which consist of a single polypeptide chain; and the type II RIPs such as ricin and abrin, which contain an A chain that is essentially equivalent to a type I RIP, as well as a lectin-like B chain, that facilitates their entry into the cytosol (Chambery et al. 2007).

**1.2.1.4. Nature of lectins**

Mature seeds are the main source of plant lectins, but they are also found in other vegetative tissues such as leaves, fruits roots but in lesser amount; (De Hoff et al. 2009). Plant lectins are secretary proteins that accumulate either in the vacuole or extracellular matrix. Most plants contain only one lectin, but in other cases, they
contain two or more biologically different lectins (Hartley & Lord 2004; Peumans & Van Damme 1998).

In nature lectins occur as dimers or tetramers with subunits of molecular weight ranging from 25-35 kDa; also there are monomeric lectins. Subunits are usually identical single polypeptide chains encoded for by different genes or members of closely related gene families; e.g. phytoagglutinins; subunits could also be distinct as for α- and β-subunits of PSL (Pisumsativum lectin) (De Hoff et al. 2009).

Some lectins are composed of subunits with different binding sites, while others contain more than one binding domain, which gives the other biological activities beside carbohydrate-binding activity (Mo et al. 2001).

1.2.1.5. Lectin-carbohydrate interaction

Lectin-carbohydrate interaction studies are of great value to improve our understanding in this area for more enhanced and better exploitation in biotechnology and other disciplines. Lectins interact with their specific ligands through hydrogen bonds, Van Der Waals forces, hydrophobic interactions and rarely electrostatic forces; coordination with metal ions also play an important role in ligand binding and lectin hemagglutination activity (Sharon 2007).

Kurt Drickamer proposed in 1988 that carbohydrate-binding activity is achieved only by limited amino acid residue and named it carbohydrate-recognition domain CRD (Sharon & Lis 2004a).

Side chains of the amino acids and the main chain groups in the CRD residue are very important in binding of sugars and creating needed forces for it (De Hoff et al. 2009; Sharon 2007).
Sharon N. reviewed that three key amino acid residues, an aspartic acid, an asparagine and an aromatic one, is essential for galactose binding in his mutational studies of the combining site residues of *Erythrina corallodendron* (Amna Elhag. 2010). The first two residues form hydrogen bonds with the hydroxyls of ligand, whereas the third interacts hydrophobically with it. They also found that an identical group of amino acid residues is involved in mannose binding by other legume lectins. They suggested that homologous lectins with distinct specificities might bind different monosaccharides by the same set of amino acid residues which are identical in their tertiary structure position but with the ligand in different orientation (Sharon 2007; Maly et al. 1985).

1.2.1.6. Biological significance of lectins

The biological function of lectins has been unknown for a long time, despite excessive studies on their structure; but different theories about their role have been denoted such as suggestion about defense role in plant. Studying the three dimensional structure, sequence homology of lectins suggested that lectins are conserved throughout the course of evolution (Sharon 2008); according to this it was suggested that lectins have a much more important biological role in plant. It is thought that lectins play an important physiological role as recognition molecules within a cell, between cells, or between organisms. Lectins are involved in the control of many biological systems (Lin et al. 2010).

Plant lectins serve as defense mechanism against insects and fungi. Legume lectins mediate the interaction between the bacteria that fix nitrogen and legume roots in symbiosis (Sharon 2007; Chan et al. 2013).

In seeds lectins serve as storage proteins, anti-predation against microbes, insects and herbivores (De Hoff at al.2009).
Mannose and N-acetylglucosamine-specific lectins on macrophages mediate the phagocytosis in animals, while β-galactosidase-specific lectins serve in organ formation and differentiation. Lectins play a vital role in lymphocyte migration to lymphoid organs and also in metastasis of cancerous cells (Ang et al. 2014a). Lectins have the ability to act as cytokines, chemokines and growth factors such as galectins and collectins (Croci et al. 2013).

Lectins located in the intracellular compartments serve in trafficking, sorting and targeting of glycoproteins, protein folding, fertilization and development; e.g. calnexin which is found in endoplasmic reticulum retain misfolded glycoproteins back into ER. Lectins function in intracellular translocation of glycoproteins (Dommett et al. 2013; Souza et al. 2013).

Animal lectins have roles in cellular regulation, migration and adhesion, phagocytosis, signal transduction, cell-cell interaction and binding of microorganisms to host cells. Mannose-binding lectin MBL is a serum lectin, member of collectins C-type lectins produced in liver, provides a third pathway of complement activation (Coelho et al. 2006).

Bacterial surface lectins function in the initiation of infection and protection against infectious agents (Sharon 2007). L-rhamnose-binding lectin in fish eggs is involved in carbohydrate metabolism, cross-linking of carbohydrate-rich proteins in the fertilization envelope, mitogenesis, lectin mediated cellular cytotoxicity, opsonization of pathogens, and has antibacterial activity (Ang et al. 2014b).

1.2.1.7. Application of lectins in biotechnology

Lectins are widely used for the isolation, identification, and characterization of different cells, they can recognize and differentiate between different cells (Amna Elhag. 2010). This characteristic along with sugar specificity are particularly useful
when studying tumor cell recognition, lymphocyte subpopulation studies, cell adhesion and localization, potentiation of host immune defense and histochemical studies of healthy and pathological conditions (Alencar et al. 2010; Peumans & Van Damme 1998).

In recent years, plant lectins have been reported to inhibit tumorigenesis and induce apoptosis in a variety of tumor cell lines. Legume lectins exhibited antiproliferative activity as well as apoptotic-inducing effects on tumorigenic cells; lectins are used in mitogenic stimulation studies, cytotoxicity and apoptosis (Chen et al. 2013).

There has been considerable interest in the ability of lectins to bind to the terminal sugars on glycoproteins and glycolipids suggesting that they are important for pattern recognition receptors. Thus, lectins are used in lectin affinity chromatography to isolate either soluble glycoproteins or membrane glycoproteins e.g. ConA or WGA-Sepharose 4B affinity matrices are commercially produced to purify glycoproteins (Ang et al. 2014b). In addition to that they are used to study cell surface glycoconjugates during cell division and throughout malignancy, purification of glycoconjugates or glycoproteins. Recently, lectins were used to prepare microarray chips for profiling global changes in mammalian and bacterial cell surface glycomes (Sahly et al. 2008).

Lectins are also used in disease diagnosis (Arshad et al. 2013). The most challenging role of drug delivery molecules is to overcome biological barriers between the site of administration and the site of action. Chemical drug delivery systems suffer from poor biopharmaceutical properties, such as poor water solubility, for this reasons the search for biological molecules started as by using of lectin for coating of some drugs to enhance their solubility. The idea of using lectins as drug delivery systems came to the table. Many reasons had brought lectins to attention; being proteins or glycoproteins and the specific and strong
binding to sugars (e.g. on cell membrane) offer the specific bio-adhesive property and binding directly to target cells offered them as potential therapeutics and/ or drug delivery systems (Assali et al. 2013). Lectins are used in coating of drugs to enhance gastrointestinal absorption (Fernandes et al. 2012).

1.2.2. Cancer

Cancer is a term, which is used to refer to a number of conditions where the cells begin to grow and reproduce in an uncontrollable way. Sometimes a cancer begins in one part of the body and then spreads to other parts of the body. This process is known as metastasis (Kleihues et al. 2002; Lewandowicz et al. 2000). Cancer occurs when the genes in a cell become abnormal and the cell divides and grows uncontrollably. Cell division is normal, but when this process is uncontrolled a mass of tissue called a growth or tumor is formed (Cancer Research UK, 2007).

Malignant tumors are cancerous and also can spread widely to other parts of the body by entering either the blood stream or lymphatic system as well as harming nearby tissues and the organs. This rapid growth of cancerous cells is known as malignant tumor. These cells can then invade and destroy healthy tissues, including organs. In addition, it can harm a number of vital organs at the same time (Merlo et al. 2006).

1.2.2.1. Types of cancers:

There are different types of cancers in the body. They include anal, bladder, breast, cervical, colon, endometrial, oesophageal, kidney, leukaemia, liver, lung, lymphoma, ovarian, pancreatic, penile, prostate, skin, stomach cancer and several others. Almost any part of the body can be infected with cancers. Cancer may be categorized based on the functions and locations of the cells from which they originate (Roberts and Rudee. 1988).
1.2.2.2. Causes of cancer

There are many causes of cancer and they include mutation of the genes that control cell growth, chemicals, radiations, preservatives etc (Merlo et al. 2006). Gene mutation is found in every cell in the body and it regulates all of its activities. Cancers are caused by damage to the DNA. The body is usually able to repair damaged DNA, but it is unable to do so in cancer cells (Goodarz et al. 2005). In most cases, people damage their DNA via their lifestyle habits, which include unbalanced diet, smoking, stress, exposure to ultraviolet radiation (UV) from the sun and to substances known as carcinogens in the environment. Some carcinogens include benzene, asbestos, formaldehyde, which are specific for skin cancers and in most cases these can be prevented with care and by following health and safety regulations. Smoking causes the majority of lung cancers, but scientists have long known that tobacco contributes to a number of other forms of the disease (Roberts et al. 1988). Each type of cancer is caused by different factors, which are well established, while others are uncertain and unknown (Goodarz et al. 2005).

1.2.3. Momordica balsamina

*M. balsamina* is a plant commonly known as Balsam apple or Bitter melon, Dragon Flower in Arabic and Aeer locally in Sudan. It’s a climber or trailer with annual stems attaining 4–5 meters in length, a plant of dry Savannah (Amna Elhag. 2010). Hutchinson,(1954) described the fruit as orange yellow, beaked, 21/2 inches in length bursting and exposing red brown seeds (Thakur et al. 2009).

This species is closely related to *M. charantia* which occurs in areas of greater rainfall (Horejsi et al. 1980). The leaves, fruits, seeds, and bark of the plant contains resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside, saponins having various medicinal importance for instance anti-HIV (Kaur et al. 2013).
The leaves are also important source of nutrients having 17 amino acids with sufficient mineral composition like potassium, magnesium, phosphorus, calcium, sodium, zinc, manganese and iron (Flyman & Afolayan 2007). High potassium content is a good source for the management of hypertension and other cardiovascular conditions (Amna Elhag. 2010). The leaves are used as soap substitute, arrow poison and fishing, the leaves as well are reported to increase milking in lactating women (Puri 2010).

The aqueous extract of the plant reduces the period pain in young girls. In Nigeria it’s used in folk medicine for treating ailments, including various digestive disorders, and asthenia (Iwalokun et al. 2001).

The whole plant powder is used for dusting over leprous and other intractable ulcers and in healing wounds. The leaf juice is given in bilious affections (Thakur et al. 2009).

Notwithstanding the emetic and purgative properties, leaves, and sometimes the fruit, are eaten in sauces and soups in the Region. In Jebel Marra of Sudan the leaves serve as a vegetable (Amna Elhag. 2010).
1.2.3.1. Classification of *M. balsamina*

From plants database (http://plants.usda.gov/core/profile?symbol=MOBA)

Kingdom: Plantae

Subkingdom: Tracheobionata

Super division: Spermatophyta

Division: Magnoliphyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Violales

Family: Cucurbitaceae

Genus: *Momordica*

Species: *balsamina*

1.2.3.2. Studies on medicinal values of *M. balsamina*

Ilango K. and his colleagues evaluated wound healing properties of hexane and methanolic extract of the fruit pulp of *M. balsamina* on excision wound model; the activity was compared with standard drug Ciprofloxacin (10 mg/kg) and Povidone Iodine Ointment (5% w/w) respectively. They found that *M. balsamina* pulp extract has better effect on wound healing (Puri 2010).

Bot Y. S. and colleagues examined Anti-HIV properties of the fruit pulp extract of *Momordica balsamina* (Bot Y. S. et al., 2007). Their results showed that the plant extract treatment significantly (P < 0.05) increased the CD4+ count when compared to the untreated peripheral blood mononuclear cells (Kaur et al. 2013).

*M. balsamina* along with two other plants were investigated for activity against multidrug-resistant *Shigella* species isolated from patients with bacillary dysentery
in Lagos by Iwalokun B. A. and colleagues (Amna Elhag. 2010). They found that *M. balsamina* possessed low shigellocidal potential compared to the other plants in the study (Iwalokun et al. 2001).

Cátia Ramalhete et al. evaluated the antimalarials with a triterpenic scaffold of the methanol extract of *M. balsamina*. They isolated three new cucurbitane-type triterpenoids, balsaminols C–E. Balsaminols were evaluated for their antimalarial activity against the *Plasmodium falciparum* chloroquine-sensitive strain 3D7 and the Chloroquine-resistant clone Dd2. Most of the compounds displayed antimalarial activity (Ramalhete et al. 2010).

Recent studies had revealed hypoglycaemic effects of leaves and fruits (Karumi and Bobboi 1999) and stem-park extract (Geidamet al. 2007) in rats, anti-inflammatory and analgesic properties of the leaf extract. Also the antimalarial activity of *M. balsamina* was confirmed in vitro and in vivo without any toxicity in healthy mice by Benoit-Vical F. and his colleagues (Oit-Vical et al. 2006). In Sudan the fruit is used for wound healing, the leaves powder as camel fodder (Amna Elhag. 2010)...
1.3. The study Rationale

Cancer is a common condition and a serious health problem all over the world, responsible for high mortality. It is estimated that 7.6 million people in the world died of cancer in 2009. In the UK alone cancer is responsible for 126,000 deaths per year (Cancer Research UK, 2007). There are many types of cancer; each type is caused by different factors, which are well established, while in other types of cancer there are no known risk factors (Goodarz et al. 2005). There are many types of cancer treatment, such as surgery, chemotherapy, radiation therapy, and many others.

A multitude of plants have been identified and used for the treatment of different diseases throughout the world, especially in poor countries. Several reports link between some plant lectins and their capabilities to inhibit growth of cancer cells, both in vitro and in vivo.

Much research has been focused on the scientific evaluation of traditional drugs from the tropical plant; *Momordica charantia* (MC), which has been commonly used as an anti-cancer agent and anti-diabetic agent (Heinrich and Bremner, 2006). Several groups of investigators have reported that treatment of a number of cancer cell lines by *Momordica charantia* related products leads to cell cycle arrest and apoptosis without affecting normal cell growth (Fang et al. 2012; Licastro et al. 1980). The lectins of *Momordica charantia* have been intensively characterized. It has been found to posses anti-tumor activity with no-to-low side effects in animals as well as in humans (Barbieri et al. 1980).

*Momordica balsamina* is a closely related species to *Momordica charantia*, being from the same Genus (see Chapter one, page 18). *Momordica balsamina* is also known as ‘Balsam apple’ or African pumpkin, is an important medicinal and nutritional plant of the Cucurbitaceae Family. 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 and fruit extracts of this plant show antiplasmodial activity and is being used against malaria in African traditional medicine. The extract of various parts of this plant shows shigelloccidal, anti-diarrheal, antiseptic, antibacterial, antiviral, anti-inflammatory, hypoglycemic and antimicrobial properties (Thakur et al. 2011). Although *Momordica balsamina* has been shown to have many medicinal activities, no lectin has been purified from it. We therefore, decided to attempt to purify and characterize *Momordica balsamina* seed lectin, and to study its anticancer effect on a group of commercial cancer cell lines.
1.4. Objectives

1.4.1. General objectives

- To isolate and purify the *Momordica balsamina* seed lectin and to study its physicochemical properties as well as its possible modulating effects on some commercial cancer cell lines.

1.4.2. Specific objectives

- To detect, isolate and purify *Momordica balsamina* seed lectin in a homogenous form to enable fine characterization.
- To study the possible modulating effects of *Momordica balsamina* seed lectin on four commercial cancer cell lines (AGS (Human Gastric Adenocarcinoma), MKN45 (Human Gastric Cancer), U87-MG (Human Glioblastoma) and ECV-304 (Human Urinary Bladder Carcinoma)).