1.1 General Introduction:

1.1.1 Cancer: Cancers are one of the leading cause of death all over the globe. WHO estimates about 70% increase in fresh cases over the next two decades. Cancer accounts for 13% of all human deaths and has become the second most common cause of mortality after cardiovascular diseases (WHO, 2015). About 2.5 million cases were reported from India with 0.5 million deaths and over 0.8 million new cases each year (Dikshit et al., 2012). Primarily, it is a life style and environmental disease with 90–95% of cases associated with life style and environmental factor and 5–10% with genetic factors (Anand et al., 2008). Some common life style factors which contribute to cancer are tobacco, radiation, stress, lack of physical activity, obesity, and infections. Cancers are disease of cell characterized by shift in the physiological mechanism which govern the cell differentiation and proliferation. They share six genotype manifestations which collectively contribute to malignant growth and modify the physiology of cells. These manifestations comprise continuous angiogenesis, lower sensitivity shown by growth-inhibitory signals, silencing of apoptotic potential, persistent replication potential, self-sufficient growth signals, and tissue invasion and metastasis (Douglas and Weinberg, 2000). Any one of the event mentioned above is obligated compensatory overpowering the apoptosis, offering support for neoplastic development. Apoptosis is a distinct form of cell death that is regulated by extrinsic or intrinsic pathways (Verma et al., 2008). Extrinsic pathway involves the execution through cell surface death receptors (TNFR1, DR4 and CD95), recruiting Fas associated death domain (FADD), caspase-8 auto activation with downstream up-regulation of caspase-3, -6 or -7 (Pal et al., 2012). The intrinsic pathway is mediated through mitochondria where the permeability transition pores, or through channel formed by Bax that release cytochrome c in cytosol leading to the activation of caspase-9 (Kumar et al., 2013).

1.1.2 The immune system

The immune system is responsible for defending the organism against attack by various infectious microorganisms, sustaining homeostasis and immune surveillance. It has developed as an integrated system of interacting cells and their products that co-ordinately identify and respond to antigenic materials (Unutmaz, 2001). The immune system may be activated by variety of stimuli, either exogenously for example by microorganisms or endogenously for example transformed neoplastic cells, that share common characteristic of being recognized as foreign by the host cell. The cellular
components that organize the framework of an immune reaction include, granulocytes, lymphocytes, mononuclear phagocytes plasma cells, mast cells, and blast cells. All these cells originate from pleuripotential haematopoetic stem cells located within the bone marrow, fetal liver, and yolk sac of the fetus. Functionally these cells may be classified as phagocytic cells, mediator cells and lymphocytes. The phagocytic cells include the mononuclear phagocytes (MNL) and poly-morphonuclear leukocytes (PMNL); the mediator cells include the mast cells, basophils, platelets; and the lymphocytes, T-cells and B-cells (Stone et al., 2010).

![Diagram of immune system cells](image)

**Figure 1.1:** Cells of immune system (adapted from Todar, 2008)

The immune system can be divided into innate and adaptive branches. Elements of the embryonic immune system continue in vertebrates as innate immunity along with an additional highly evolved system of specific responses called adaptive immunity. Both of these systems work in concert to offer a high degree of defense for vertebrate species. Both branches impact each other and are in turn shaped by their environment. Both innate and adaptive immune response depend on the capability of the immune system to differentiate between self and non-self molecules. Self molecules are those components that can be detected as a part of organism's own body and can be differentiated from foreign materials by immune system. On the other hand, non-self molecules are recognized as foreign materials. The non-self molecules are designated
as antigen, that bind to a specific molecules called receptors and elicit an immune reaction (Litman et al., 2010).

**Table 1.1: Components of immune system**

<table>
<thead>
<tr>
<th>Innate immune system</th>
<th>Adaptive immune system</th>
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</thead>
<tbody>
<tr>
<td>It elicits a non-specific immune response</td>
<td>It elicits an antigen or pathogen specific immune response</td>
</tr>
<tr>
<td>An immediate maximal response is expressed on antigenic exposure</td>
<td>There is a lag time between antigenic exposure and maximal response</td>
</tr>
<tr>
<td>It contain humoral and cell mediated components</td>
<td>It also contain humoral and cell mediated components</td>
</tr>
<tr>
<td>It does not result in any immunological memory</td>
<td>Exposure leads to immunological memory for that particular antigen</td>
</tr>
<tr>
<td>Found in all forms of life</td>
<td>Found only in jawed vertebrates</td>
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</table>

Following the stimulation of the immune system, a spectrum of cellular and humoral events occur that comprise the non-specific and specific immune response. The non-specific immune responses represent the host’s initial encounter with the foreign material. These responses are characterized by inflammatory reactions and phagocytosis. The phagocytic cells engulf any foreign material they come across, and eliminate it by intracellular digestion. On the other hand, the specific immune responses depends upon the exposure to foreign material, and the subsequent recognition and reaction to it. These responses are carried out by the immune-competent cells, the lymphocytes, which individually and collectively express the cardinal properties of the immune response, i.e. specificity, memory, recognition and amplification (Charles et al., 2001).

Lymphocytes are responsible for the primary recognition of the antigen and have the ability to react specifically to it and liberate specific cell products. The detection of various immunological markers on the lymphocyte membrane as well as the functional characteristics of the lymphocytes have permitted identification of two distinct populations of the lymphocytes called T-cells and B-cells (LeBien and Tedder, 2008). The T-lymphocytes are thymus derived or thymus influenced during their development and participate in cell mediated immunity (delayed type hypersensitivity, allograft
rejection, antitumor immunity and cellular defense against fungi and intracellular pathogens). They respond to the foreign material through surface receptors identified as heterodimers composed of α and β chains. B-lymphocytes (B-cells) are responsible for humoral immunity which is expressed by the production of specific circulating plasma proteins termed as antibodies or immunoglobulin. The B-lymphocytes are the cells that initially respond to the foreign material, either through immunization or infection. Following the interaction and recognition of the foreign material, the T-cells are stimulated by the antigen to undergo a transformation to the blast cells that divide, proliferate and differentiate into sensitized T-cells. These cells together take part in cell mediated reactions either directly or through liberation of lymphokines. Macrophages are the important cells of the immune system which play a significant role in host defense mechanisms (Mogensen, 2009). Binding of bacteria to macrophages results in the production of TNF-α. It acts in an autocrine manner to induce the expression of i-NOS gene resulting in the production of nitric oxide (NO). NO released in the cell is toxic and can kill micro-organisms in the vicinity of the macrophage. The uptake of antigens by macrophages is the first step in the processing of antigen leading to the production of circulating antibody. The processed antigen is presented to the antigen-specific T and B cells. The T-helper cells activate B-cells to proliferate and differentiate into plasma cells which secrete specific immunoglobulin. The T-suppressor cells then inhibit the B-cell differentiation, proliferation and activate macrophages to become suppressor cells to shut the reaction (Charles et al., 2001).

The immune system is a multicellular system involving the interaction of foreign substances with a wide variety of cell types. At least three distinct cell populations are required for maximal antibody production to most antigens; T-cells, B-cells and macrophages. The uptake of antigens by macrophages is the first step in the processing of antigen leading to the production of circulating antibody. The macrophages do not recognize the antigen specifically as foreign but non-specifically processes it so that it becomes more palatable for a specific antigen recognition cell. Memory is a representative feature of the immune system. Following stimulation by the antigen, the lymphocytes undergo proliferation and differentiation into plasma cells that synthesize and secrete antibody (humoral immunity) or into specifically sensitized lymphocytes that have ability to react specifically with the antigen (cellular immunity). In addition, a specific cell or cell line with particular property of reacting on second contact with
the original antigen by a more rapid and increased proliferation and differentiations is also produced (memory cells). A second contact with the same or closely related antigen stimulates a more rapid reaction with the production of a greater specific immune response (Swain, 2010).

1.1.3 The immune system and cancer

The understanding that in cancer progression, the immune system plays a dual role, has led to the development of targeted immunotherapies (Zigler et al., 2013). The immune system has potential to recognize and destroy tumors, and thus function as a prime defense against cancer. Immune system plays three main roles in tumor prevention; elimination or suppression of viral infection; prevention of the formation of an inflammatory setting conducive to tumorigenesis through prompt resolution of inflammation and elimination of antigens; and recognition of cancerous or precancerous cells and their eradication (cancer immune surveillance) (Swann and Smyth, 2013). In spite of cancer immune surveillance, tumors can develop in the presence of an active immune system, and therefore the concept of cancer immunoediting was put forward to explain the role of the immunity in tumor development (Kim et al., 2007). The concept can be divided into three stages viz. elimination, equilibrium, and escape (Vesely et al., 2011; Stewert and Abrams, 2008) (Figure 1.2). The elimination stage of cancer immunoediting is precisely similar to that of tumor immune surveillance, where the immune system identifies and eradicates tumor cells that have settled as a result of unsuccessful intrinsic tumor suppressor mechanisms. The equilibrium phase triggers immune-mediated tumor latency but is poorly understood as this phase has been difficult to induce in mice and is defined only anecdotally in humans (Schreiber et al., 2011). The escape phase, can occur through different mechanisms including: increased resistance or survival, reduced immune recognition and development of an immunosuppressive tumor microenvironment (Vesely et al., 2011)
In cancers, the metabolic and immune system of the patient becomes severely suppressed, partially because of the systematic diminishing brought by the cancer process and partially because of the damaging, poisonous effects of conventional chemotherapies. If the immune system of the body is compromised, there are much higher chances of development and spreading of cancer or other disorders. The individuals having a strong immune system have a significantly better chance of eliminating carcinogens before cancerous activity starts. Cancer and drugs/radiations used for its treatment, can significantly weaken the body's immune system, affecting the blood cells that protect against disease and germs. As a result, cancer patient cannot fight infection, foreign substances, and disease as well as a healthy person. Potentiation of the host defense mechanism may result in the stimulation of immune cells that are extremely important for the maintenance of homeostasis (Ooi and Liu, 2000). Therefore, immunomodulation mediated anticancer therapy has generated significant interest in scientific community.

1.1.4 Rasayana concept of Ayurveda
Plant and animal products have been used effectively for the treatment of pathological states in humans since ancient times. Every country in the world has enlisted various
indigenous herbal remedies according to the disease and human requirements. In Indian System of Medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans (Ravishankar and Shukla, 2007). Ayurveda is one of the traditional system of medicine practiced in India and Sri Lanka, and can be traced back to 6000 BC. Ayurveda, literally the "science of life and longevity" in ancient Sanskrit, is the one of the oldest healing system, based on lifestyle, diet and herbs. Ayurvedic pharmacology classifies medicinal plants into multiple groups according to their action. One of these is rasayana. The word rasayana means path that rasa takes (rasa: the primordial tissue or plasma; ayana: path). It is believed, in Ayurveda, that the qualities of the rasa-dhatu influence the health of other dhatus (tissue) of the body. Hence any medicine that improves the quality of rasa (rasayanas) may strengthen or promote the health of all tissue of the body. The rasayana plants are said to possess properties that prevent ageing, re-establish youth, strengthen life and brain power and prevent disease (Sharma and Bhagwan, 1976; Ghanekar, 1981), all of which imply that they increase the resistance of the body against any onslaught. Traditionally, these agents are used against a plethora of seemingly diverse disorders with no pathophysiological connection according to modern medicine.

*Brugmansia suaveolens* (*B. suaveolens*) and *Nicandra physalodes* (*N. physalodes*) are the plants from Himalyan regions which are not fully explored. Both of *B. suaveolens* and *N. physalodes* are members of Solanaceae family and this family is known for their high withanolide contents which are reported to have promising immunomodulatory and anticancer activity (Zhang et al., 2012). These compounds have been evaluated for immunomodulatory activity by observing their effect on antibody production, T-cell and B-cell activation, and cytokine production from spleenocytes (Bhat et al., 2006). This study is designed to explore new bioactive compounds from these plants which may have better selectivity for both immunomodulation and immunomodulation mediated anticancer activity.
1.2 Aim and objectives
The present study has been designed to investigate the anticancer and immunomodulatory potential of the phytoconstituents from *Brugmansia suaveolens* and *Nicandra physalodes*.

Objectives

1.2.1 Biological evaluation of phytoconstituents from selected plants of Solanaceae family for immunomodulatory activity.

1.2.2 Biological evaluation of phytoconstituents from selected plants of Solanaceae family for anticancer activity.

1.2.3 Molecular mechanism of immunomodulation mediated anticancer activity.