1. INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. India has a rich history of using plants for medicinal purpose. Several plant products are known to exhibit creditable medicinal properties for the treatment of various ailments. In developing countries, all over the world, 80 percent of population continues to use traditional medicine in primary health problems (Yadav et al., 2010). In the past decade, research has been focused on scientific evaluation of dietary plants and preparation of medicine of plant origin. Pumpkin is one such plant, cultivated throughout the world for use as vegetable as well as medicine (Jia et al., 2003).

Pumpkin (Cucurbita maxima) is a common vegetable, widely distributed in India, Bangladesh, Australia, Germany, Japan, China, USA, Brazil and other countries. Cucurbita maxima (Pumpkin) can be found in many shapes, sizes and colours. Due to the medicinal properties of pumpkin the whole part of the vegetable can be used for human consumption. Pumpkin has been used for pies, curry, soups, stews, jam, sweet, marmalade, beverage, baby food, ice-cream, instant pumpkin kofta and breads. It has to be processed in different ways for human consumption such as fried, frozen, dried, candies or pickled. Leaves, stem, seeds and roots have high food value and provide source of oil and raw material for variety of products. The leaves and flowers of the plants are widely cooked as vegetable soup.

The pumpkin seed is valued in relation to its nutritional points. The four fatty acids present in significant quantities are palmitic, stearic, oleic, and linoleic acids (Stevenson et al., 2007). Pumpkin seeds contain 39.25% crude protein, 27.83% crude oil, 4.59% ash and 16.84% crude fiber; the corresponding values for the kernels were 39.22%, 43.69%, 5.14%, and 2.13%, respectively. The saturated fatty acid content was 27.73% and comprises of 16.41% palmitic acid and 11.14% stearic acid. The unsaturated fatty acid value was 73.03% and consists mainly of 18.14% oleic acid and 52.69% linoleic acid. While considering the lipid and protein content, fatty and amino acid compositions, the pumpkin seed kernels appear to be quite promising for commercial exploitation (Mohamad, 2004).

Pumpkin seed oil is rich in antioxidants and beneficial as nutritional supplements such as essential fatty acids including linoleic and linolenic, carotenes, lutein, gamma and P-tocopherols, phytoserols, chlorophyll, selenium and zinc (Lee, 2006; Williams et al., 2006).
Pumpkin seeds are typically rather flat, asymmetrically oval, and light green in color inside a white hull (www.wikipedia,pumpkinseed, assessed on 10/10/2011). The pumpkin seeds possess dietary and medicinal qualities besides being the source of good quality edible oils. Pumpkin seed oil has been used traditionally as medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil, and America (Fu et al., 2006).

Processing techniques causes important changes in the biochemical, protein, nutritional and sensory characteristics of seeds. Processing methods, such as soaking, germination, roasting and autoclaving has been reported to improve the nutritional properties of plant seeds (Yagoub and Abdella, 2007). During the germination there are certain changes that could occur in the quality and type of nutrients within the seed. These changes can vary depending on the type and variety of the seed and the conditions of germination. Cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients. It also improves palatability; enhance the digestibility and nutritive value (Oboh et al., 2000; Ramakrishna et al., 2006).

The factors influence the nutrient content of the food, includes the genetic make-up of the plant, the soil in which it is grown, use of fertilizers, pesticides, prevailing weather, and maturity at harvest, packaging, storage conditions and method of preparation for processing. The storage conditions and handling after processing will also affected the nutritive value of the food. The effect of food processing on nutrient content will depend on the sensitivity of the nutrient to the various conditions prevailing during the process, such as heat, oxygen, pH and light. The nutrient retention may vary with the combination of conditions, such as the characteristics of the food being processed, and the concentration of the nutrients in the food. Heating can be both beneficial and detrimental to nutrient content of foods. It generally improves the digestibility of foods, making some nutrients more available (Morris, 2004). Studies have shown that thermally processed foods, especially fruits and vegetables, exhibited higher biological activities due to various chemical changes undergone during heat treatment (Kim et al., 2000; Dewanto et al., 2002).

One potential food application for pumpkin seed flour is its use in composite flours for the production of bakery products, such as bread. Bread is an important ready-to-eat product which is becoming increasingly popular in hotels, restaurants, canteens and in households. The bread is a fast and convenient food based on wheat. It is one of the major products of baked foods and is consumed worldwide (Bakke and
The bread is an ideal product that can serve as a functional food, since it is daily used by a large population throughout the world (Martin, 2004; Sluimer, 2005). Bread products are well accepted worldwide because of the low cost, ease of preparation, versatility, sensory attributes and nutritional properties. Bread in human nutrition is not only a source of energy, but also a supplier of irreplaceable nutrients for the human body. It provides little fat, but high quantities of starch and dietary fiber as well as cereal protein. Apart from that, bread contains the B-group vitamins and minerals which are mostly magnesium, calcium and iron. The sensory scores for appearance, texture and flavour of breads have been reported to decrease by the incorporation of non wheat flours in wheat flours (Shittu et al., 2007).

Addition of pumpkin seeds with wheat flour help to increase the protein content, improve the nutritional quality of bread, especially amino acid content. Baking characteristics, such as loaf volume and dough mixing, can also be improved. The quality of bread is based on the physical, chemical and sensory properties of the bread. The optimum proportion of pumpkin seed powder in to wheat flour can be determined by the Response Surface Methodology (RSM). RSM is more efficient than traditional experimental procedures because it decreases the time and cost required to determine the optimum product.

Response surface methodology (RSM) has been shown to be useful tool in the food field, for the development of new products and processes. RSM is a statistical technique that uses quantitative data to determine and simultaneously solve multivariate equations, which specify the optimum product for a specified set of factors through mathematical models. These models consider interactions among the test factors and can be used to determine how the product changes with changes in the factor levels. RSM is important applications in the design, development, and formulation of new products, as well as in the improvement of existing product design (Myers and Montgomery, 2002).

Pumpkin seed (*Cucurbita maxima*) has been claimed to combat benign prostatic hyperplasia. *Cucurbita maxima* seed oil contains essential fatty acids that help to maintain healthy blood vessels, nerves and tissues, (Rachel, 2008). The medicinal properties of *Cucurbita maxima* include anti-diabetic, antioxidant, anticarcinogenic, and anti-inflammatory (Yadav, 2010). The elevated levels of serum testosterone may be one of the mechanisms underlying the effect of squalene in pumpkin seeds on improvement in libido and semen quality and the reduction in serum leptin (Bjorbaek and Kahn, 2004).
Pumpkin seeds will contribute to a good amount of essential fatty acids Omega-3 and Omega-6. First, it provides very important advantages for the health such as the diminution of the cholesterol, the hypertension, the inflammation in arthritis or breast cancer. The Omega-6, besides being very beneficial also for the circulatory apparatus, has properties for the treatment of the negative symptoms of the pre-menstrual syndrome (www./botanical online.com/index. accessed on 30/5/2011). They maintain the fluidity of cellular membranes, aid in producing and balancing hormones, and play an essential role in managing healthy fluid levels. Availability of more zinc might reactivate the normal functioning of receptors (Bataineh et al., 2002).

Pumpkin seeds have many magical functions in human health. It helps to cure many diseases but two burden leading diseases are depression and hypercholesterolemia which leads to cardiovascular disease. Phytosterols present in pumpkin seeds have tremendous cholesterol lowering properties and hence, are added to butter substitutes, making an ideal eatable for people suffering from this problem. Phytosterols work towards stimulating the excretion of cholesterol and blocking its absorption by the walls of the intestines. Apart from lowering cholesterol, the presence of phytosterols in pumpkin seeds is useful for enhancing heart health and reducing the risk of heart. Pumpkin seeds contain the compound tryptophan which is necessary to battle the feelings of depression.

According to World Health Organization (2004), “Depression was the fourth largest contributor to the diseases burden in 1990 and is expected to be the second largest after ischaemic heart disease by 2020”. According to a study of the World Health Organization, depression is currently the fourth leading cause of morbidity and, within a ten-year period, it might rank second among the disorders affecting productive life (Fava and Kendler, 2000). Depression has been associated with increased risky behaviours, non compliance to treatment, higher risk for co-morbid disorders and shortened survival (Horberg et al., 2008; Farinpour et al., 2003; Cook et al., 2004).

World Mental Health Survey Initiative (WHO) has said that India has the highest rate of major depression in the world. The average lifetime rates of depression, according to WHO study, were found to be 14.6 percent in ten high income countries, and 11.1 percent in eight low- to middle-income countries. But lifetime incidents of what was identified as Major Depressive Episodes (MDE), were highest among Indians at 35.9 percent, while China was at the lowest at 12 percent average percentage of MDE was however, considerably higher in high-income countries at 28.1 percent, compared to
19.8 percent in the low- to middle-income countries (Bromet et al., 2011). Depressive disorders are associated with intense suffering, high morbidity rates, and increased mortality (Fava and Kendler, 2000). Major depressive disorder is defined by episodes of depressed mood lasting for more than 2 weeks accompanied by additional symptoms including disturbed sleep and appetite, reduced concentration, excessive guilt, and suicidal thoughts (Mill and Petronis, 2007).

Symptoms of depression includes agitation, restlessness, and irritability, dramatic change in appetite, weight gain or loss, very difficult to concentrate, fatigue, lack of energy, feelings of hopelessness and helplessness, feelings of worthlessness, self-hate, and guilt, becoming withdrawn or isolated, loss of interest or pleasure in activities that were once enjoyed, thoughts of death or suicide and trouble sleeping or excessive sleeping (Fava and Cassano, 2008). Sleeping (Fava and Cassano, 2008). The following may play a role in depression: alcohol or drug abuse, certain medical conditions, including underactive thyroid, cancer, or long-term pain, certain medications such as steroids, sleeping problems, stressful life events such as: breaking up with a boyfriend or girlfriend, failing a class, death or illness of someone close to you, divorce, childhood abuse or neglect, Job loss and Social isolation common in the elderly (Zieve, 2011).

Depression is caused by a combination of genetic, biological, environmental, psychological factors and chemical imbalance. A chemical 5-hydroxytryptophan (5-HTP) made from tryptophan regulates behavior. It can have a positive effect on sleep, anxiety, mood, appetite and pain. For mild to moderate depression, 5-HTP can be an effective treatment. It increases the serotonin in the brain, which affects mood. Pumpkin seeds are a dietary source of tryptophan, which the body uses to make 5-HTP (http://www.siemens.com/answers, accessed on 09/08/10). Depression is a highly frequent psychiatric disorder with a lifetime prevalence of 17%, being twice as prevalent among women where compared with men. Onset of depression usually occurs in the third decade of life, but the disorder can affect individuals at any age. It is a recurring condition and around 20-25% of patients become chronically ill (Fava and Kendler, 2000).

Tryptophan is an essential amino acid present in pumpkin seed, and 5-hydroxytryptophan (5-HTP) is the intermediate metabolite of tryptophan in the formation of the neurotransmitter serotonin. Both tryptophan and 5-HTP are promoted as treatment for depression. The most common nutritional deficiencies seen in mental disorder patients are of omega-3 fatty acids, B vitamins, minerals, and amino acids that
are precursors to neurotransmitters (Eby and Eby, 2006). As reported in several studies, the amino acids tryptophan, tyrosine, phenylalanine, and methionine are often helpful in treating many mood disorders, including depression (McLean et al., 2004; Rot et al., 2006). Tryptophan is a precursor to serotonin and is usually converted to serotonin when taken alone on an empty stomach. Therefore, tryptophan can induce sleep and tranquillity and restore serotonin levels leading to diminished depression. Since research in humans for depression is limited, animal models of depression have been developed, whereas many symptoms of depression cannot be easily measured in laboratory rodents (e.g. depressed mood, feelings of worthlessness, suicide tendency). However, some behavioral tests have been shown to be very effective to evaluate depressive symptoms and are classically used to predict the antidepressant effect of new medications. The existence of numerous behavioral tests to measure depression in rodents reflects the heterogeneity of depressive like symptoms. In this way, forced swimming test and tail suspension test are classical paradigms used to evaluate behavioral despair.

The two models are Forced Swimming Test (FST) and Tail Suspension Test (TST) reflects a state of despair which can reduce by several agents who are therapeutically effective in human depression (Hosseinzadeh and Madanifard, 2005). The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail (Vogel, 2002). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility.

Hypercholesterolemia is a significant risk factor for cardiovascular diseases (CVDs). According to WHO (2002), cardiovascular diseases (CVDs) will be the largest cause of death and disability by 2020 in India. In 2020 AD, 2.6 million Indians are predicted to die due to coronary heart disease which constitutes 54.1% of all CVD deaths. Nearly half of these deaths are likely to occur in young and middle aged individuals (30-69 years). In humans, hypercholesterolemia is often due to high serum Low Density Lipoprotein (LDL) cholesterol levels, which are generally atherogenic. Atherosclerosis is the pathological basis for most CVDs and is often characterized as the progressive accumulation of lipids in the vessel wall. The subsequent rate of
Atherogenesis is positively associated with the severity of associated risk factors including serum cholesterol levels (Badimmon, 2011).

Hypercholesterolemia is the presence of abnormally high levels of cholesterol in the blood, and it has reached epidemic proportions worldwide. In 2008, the highest serum total cholesterol concentrations worldwide were reported in Australia, North America, and Western Europe (Farzadfar, 2011). This disorder may result from a genetic lipoprotein metabolism deficiency, or as a consequence of an underlying systemic disease (Steinberg, 2005; Thomason et al., 2007). Hypercholesterolemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Triglycerides (TG) with a concomitant decrease, in the concentrations of High Density Lipoprotein Cholesterol (HDL-C) in the blood circulation (Kaliora and Dedoussis, 2006).

Globally, one third of ischaemic heart disease is attributable to high cholesterol. Raised cholesterol levels (Zhang et al., 2010) increase the risks of heart disease and stroke (Ezzati et al., 2002). Raised total cholesterol is a major cause of disease burden in both the developed and developing countries as a risk factor for ischaemic heart disease and stroke in both sexes (Meyer et al., 2001). The prevalence of total cholesterol increased noticeably according to the income level of the country. In low-income countries, around a quarter of adults had raised total cholesterol in lower-middle income countries this rose to around a third of it.

Hypercholesterolemia can be modified by therapeutic dietary changes including lowering cholesterol by diet or supplementing the diet with certain nutrients such as phytosterol and polyunsaturated fatty acids. The use of alternative medicines and especially the consumption of phytochemical have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use (NCEP, 2002).

Antioxidants, selenium and many phytosterol which helps to reduce the risk of arteriosclerosis, heart disease, stroke and cancer and regulate cholesterol levels. Phytosterol in pumpkin seed have been proven to reduce LDL cholesterols, which can often lead to heart disease. Of the nuts and seeds typically consumed as snack foods, pistachios and sunflower seeds were richest in phytosterol (270-289 mg/100 g), closely followed by pumpkin seeds (265 mg/100 g) (Philip, 2005).
Recently increased attention has been focused on the utilization of under-utilized agricultural products, as well as byproducts and wastes from food processing to produce food and feed. Such utilization would help maximize available resources and minimize waste disposal problems.

Keeping in view the medicinal and nutritional benefits of pumpkin seed, present study was undertaken to improve the nutritional and functional properties of the seed by different processing, evaluate the antidepressive and hypocholesterolemic effect of processed pumpkin seeds by the supplementation (extract) in rats and in human through optimized germinated pumpkin seeds bread. Therefore, investigator planned on the basis of above mentioned lines to explicitly the following objectives:

**Objectives**

1. To study the effect of processing techniques on proximate composition, mineral composition, amino acid composition, fatty acid profile and physical properties of pumpkin seeds powder.
2. To evaluate the antidepressant effect of processed pumpkin seeds aqueous extracts on selected animal model (by using behavioral animal models).
3. To investigate the hypocholesterolemic effect of processed pumpkin seeds powder extract on selected animal model.
5. To assess the antidepressant activity of optimized germinated pumpkin seed bread on selected human subjects.
6. To investigate the hypocholesterolemic activity of optimized germinated pumpkin seeds bread on selected human subjects.