Synopsis of the thesis submitted for the award of Ph.D., degree in Biochemistry of the University of Mysore, India.

Title: Studies on Phytochemicals and Biological Properties of Bitter Cumin *Centratherum anthelminticum* (L.) Kuntze

Background

Human health is inextricably linked with diet and in the last few years research teams have been able to demonstrate the health beneficial effects of food components, spices and medicinal herbs that are a part of the traditional diet. There has been an explosion of research geared towards understanding precisely how diet can affect normal physiology and health of human beings. One of the most remarkable features of medicinal plant research of the last decade is the enormously increasing interest towards the biological activities of phytochemicals such as phenolic compounds including flavonoids. Flavonoids and other phenolics have been suggested to play a preventive role of chronic diseases such as cancer and heart diseases.

Spices have occupied an important place in the lives of people since ancient times. They have been considered indispensable in flavouring, seasoning of foods, flavouring of beverages, in perfumery, cosmetics and medicines. The medicinal activity of spices and spice extracts including hypocholesterolemic, hypoglycemic, antiinflammatory, antimicrobial, antithrombotic, antimutagenic, anticarcinogenic etc., are attributed to their bioactive molecules such as polyphenolic compounds, phenolic glycosides, essential oils, phytosterols etc.,

Cumin is one of the most popular spices all over the world especially Latin America, North Africa, Europe and all over Asia. There are three major varieties of cumin, Normal cumin (*Cuminum cyminum*), Black cumin (*Nigella sativa*) and Bitter cumin (*Centratherum anthelminticum*).

Bitter cumin (*Centratherum anthelminticum* (L.) Kuntze) belongs to the family Asteraceae. It is locally called ‘Kali-ziri’ or ‘Somraj’. It is distributed throughout Indian subcontinent and also cultivated in Sri Lanka. The seeds have a hot, sharp, bitter taste, acrid, astringent to the bowls. The seeds are reported to possess febrifugal, alterative, antihelmenthic, antiulcer, antiphlegmatic, cardiac, diuretic and digestive
properties. In Ayurvedic medicine it is used to treat skin diseases such as leucoderma. In Yunani system of medicine the seeds are used as purgative and for treatment of asthma, hiccough, inflammatory swellings and itching of the eyes. On the Malabar coast an infusion of the seeds is given for the coughs and against flatulency.

There is little scientific data on phytochemicals and their beneficial properties of bitter cumin variety. The main aim of this research programme is to isolate and characterize the bioactive phytochemicals from bitter cumin and study their biological properties in suitable model systems. The main objectives of this study are

1) Isolation and characterization of bioactive phytochemicals from *Centratherum anthelminticum* (L.) Kuntze seeds.

2) To study the biological effects of *Centratherum anthelminticum* (L.) Kuntze phytochemicals on antioxidant, antidiabetic and antimicrobial properties in suitable model systems.

The thesis comprises of five chapters. Chapter I present general introduction about cumin varieties and objectives and scope of the present investigation.

Chapter 2 deals with proximate composition, total phenol content, isolation, characterization and quantification of phenolic compounds from Bitter Cumin (*Centratherum anthelminticum* (L) Kuntze) seeds. The total phenol content of different extracts of bitter cumin was determined in terms of gallic acid and tannic acid equivalents. Aqueous methanol acetone extract of bitter cumin (AMAEBC) showed highest phenolic content among different solvent extracts. The phenolic compounds in AMAEBC was determined and quantified by LC-MS. Interestingly bitter cumin seeds contained a mixture of phenolic compounds such as gallic acid, protocatechuic acid, ellagic acid, caffeic acid, ferulic acid, quercetin and kaempferol.

Chapter 3 describes the antioxidant activity of bitter cumin seed. The antioxidant activity of phenolic compounds of bitter cumin seed was determined by various *in vitro* model systems. The antioxidant assays adopted in this study are phosphomolybdenum reducing assay, potassium ferricyanide reducing method, DPPH radical scavenging, ABTS* radical scavenging, superoxide radical scavenging, soybean lipoxygenase dependent lipid peroxidation, rat liver microsomal lipid
peroxidation, egg lecithin liposomal model system and oxidative DNA damage. AMAEBC showed a wide range of antioxidant activity by scavenging or neutralizing radicals such as DPPH• radical, ABTS• cation radical, hydroxyl ('OH), lipid peroxy radical (LOO•) and superoxide anion (O2•−) and also inhibited oxidative DNA damage. The phenolic acids such as ferulic acid, caffeic acid, gallic acid, ellagic acid, protocatechuic acid and flavonoids such as quercetin and kaempferol found in bitter cumin seeds are reported to have good antioxidant activity. Hence, the phenolic compounds present in bitter cumin could be responsible for its antioxidant activity.

Chapter 4 describes antidiabetic activity of Bitter Cumin. The antidiabetic activity of AMAEBC was tested in \textit{in vitro} and \textit{in vivo} model systems. One of the therapeutic approaches for management of diabetics is decreasing the postprandial hyperglycemia by retarding the absorption of glucose in the small intestine. Hence the modulatory effect of AMAEBC on carbohydrate hydrolyzing enzymes such as intestinal \(\alpha\)-glucosidase and human salivary \(\alpha\)-amylase has been studied. AMAEBC inhibited salivary \(\alpha\)-amylase in a concentration dependent manner with an IC\(_{50}\) value of 185.5 ± 4.9 µg. Further, AMAEBC inhibited rat intestinal sucrase, maltase and PNP-glycoside activity at microgram concentrations with IC\(_{50}\) values of 34.1 ± 3.8 µg/mL, 62.4 ± 4.5 µg/mL and 500.5 ± 11.9 µg/mL respectively. The inhibitory effect of AMAEBC against sucrase was about 2 and 14 times higher than maltase and PNP-G (p-nitrophenyl glucopyranoside) hydrolysis activity respectively. Further, inhibitory effect of AMAEBC on sucrase and maltase activities is found to 8 and 32-fold higher than DL-catechin, but ≈15 and 624-fold lower than synthetic therapeutic drug acarbose. The higher inhibitory activity of AMAEBC compared to DL-catechin may be because of the additive activity of an array of phenolic compounds present in bitter cumin seeds. The enzyme kinetic studies on \(\alpha\)-glucosidase inhibition by AMAEBC showed a non-competitive type of inhibition. The antihyperglycemic effect of AMAEBC was examined on \textit{in vivo} maltose tolerance test in rats. Oral feeding of AMAEBC (50-200 mg/kg body weight) significantly reduced the postprandial plasma glucose levels comparable to that of acarbose. The results demonstrate that AMAEBC may inhibit digestion of carbohydrates in rat intestine leading to a decrease in postprandial plasma glucose level. The fasting blood glucose level of streptozotocin (STZ) induced diabetic rats after AMAEBC treatment for 8 days was reduced by 15.23% when compared to control group. In an another experiment of low STZ and high fructose induced hyperglycemia, AMAEBC caused 13.34% and 19.33% decrease in fasting blood glucose level at 25 mg/kg b.wt and 100 mg/kg b.wt.
respectively. These studies clearly show that bitter cumin extract has antihyperglycemic property by modulating the activity of carbohydrate metabolizing enzymes.

Chapter 5 describes the antimicrobial activity of Bitter Cumin. Antibacterial activity of cumin extract was tested against food-borne pathogenic and spoilage bacteria viz., Bacillus subtilis, Bacillus cereus, Enterobacter spp., Escherichia coli, Listeria monocytogenes, Staphylococcus aureus and Yersinia enterocolitica by agar diffusion method. Three bacterial species namely Bacillus cereus, Bacillus subtilis and Staphylococcus aureus were found to be highly sensitive and showed significant inhibition of the growth in presence of AMAEBC. Enterobacter spp. and Listeria monocytogenes were moderately inhibited while Escherichia coli and Yersinia enterocolitica were not sensitive to bitter cumin extract. Minimum Inhibitory Concentration (MIC) of B. cereus, S. aureus and L. monocytogenes were determined and found to be 50 ± 7 µg, 260 ± 18 µg and 700 ± 42 µg respectively.

Bitter cumin is a cumin variety which is not used in food preparations due to its bitter taste. However, bitter cumin is used extensively in Ayurvedic and traditional medicine. The present research study demonstrated that the bitter cumin seeds contain an array of bioactive phenolic compounds with a potent antioxidant, antihyperglycemic and antimicrobial activities and thus provide data to supports its usage in ayurveda and traditional medicine. Bitter cumin can be further exploited as a herbal medicine and health related herbal products for the management of different diseases.