EVALUATION AND DEVELOPMENT OF BEVERAGES AND DESSERTS SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

SUBHASREE. B

(16PFN021)

A THESIS SUBMITTED TO THE

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE- 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

APRIL 2018

EVALUATION AND DEVELOPMENT OF BEVERAGES AND DESSERTS SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

By

SUBHASREE. B

(16PFN021)

A THESIS SUBMITTED TO THE

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE- 641043.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

APRIL, 2018

Certified as a Bonafide Research Work

Signature of the Supervisor

Signature of the Head of the Department

Acknowledgement

ACKNOWLEDGEMENT

First and foremost the investigator expresses her deep sense of gratitude to **God Almighty** for showering His blessings on her who graciously blessed her with good health, strength and wisdom, to complete the study. She thanks Him for blessing her with such great and wonderful parents to guide her.

The investigator expresses her heartfelt thanks and deep sense of gratitude to **Padmashri Dr.P.R. Krishnakumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing the infrastructural facilities for the smooth conduct of the study.

The investigator expresses her reverential gratitude to **Thiru. T.S.K. Meenakshisundaram**, **M.A., M.Phil., Ph.D.,** Managing Trustee, Sri Avinashilingam Education Trust Institutions, Coimbatore, for providing the opportunity to conduct the study.

The investigator owes her special thanks and gratitude to **Dr. (Tmt.)PremavathyVijayan, M.Sc., M.Ed., Dip.Spl.Edn, M.Phil., Ph.D (Avinashilingam)**, Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for the amenities provided for the successful completion of the study.

The investigator records her sincere gratitude to **Dr. (Tmt.)S. Kowsalya, M.Sc., M.Phil., Ph.D.,** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing all help in the smoth conduct of the study.

The investigator owes her heartfelt thanks and deep dense of gratitude to **Dr. (Tmt.) N. Vasugi, M.Sc., M.Phil., Ph.D.,** Dean, Faculty of Home Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her kind support and encouragement for the conduct of the study.

The investigator expresses her special thanks and sincere gratitude to **Dr. (Tmt.) A.Thirumani Devi, M.Sc., M.Phil., Ph.D.,** Professor and Head, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for the keen interest,

valuable help, concern and encouragement which helped in the successful completion of the study.

The investigator expresses her special thanks and sincere gratitude to **Dr. (Tmt) M. Amirthaveni, M.Sc.,Dip.Ed. M.Phil., Ph.D.,**Former Professor and Head, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for the immense support in the conduct of the study.

The researcher is deeply indebted and it gives her an immense pleasure and pride to offer profound gratitude to her guide **Dr. (Tmt) R. Balasasirekha, M.Sc., PGDCA., M.Phil., Ph.D.** Assistant Professor, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her inspiring, ceaseless and dynamic guidance, supportive wisdom, continued motivation and enduring support from the initiation to the completion of the study.

The investigator expresses her heartfelt thanks to **Tmt. S. Radhadevi, M.Sc. (Kerala), M.Phil, (Madras)**, Associate Professor in Statistics, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her valuable suggestions rendered in Statistics throughout the study.

The investigator owes her sincere thanks to all the **Staff Members** of the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for being supportive and understanding.

She also thanks all the student volunteers of the study without whom the study would have been incomplete. No words are sufficient to express her deep sense of gratitude to her beloved and respected **Parents, Grandmother, Sister and Family members, Friends, Hostelmates and Roommates** for their affection, care, blessing and co-operation in all walks of her life.

The researcher is grateful to each and every soul who had helped her in one or the other way in making this study a great success.

Contents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

Review of Literature

Methodology

Results and Discussion

Summary and Conclusion

Bibliography

Appendices

I. INTRODUCTION

Even in this high-tech age, the low-tech plant continues to be the key to nutrition and health

- *Jack Weatherford*

People living in many parts of the world are consuming an average of more than 500 calories per day from added sugar alone. Over the past 50 years, consumption of sugar has tripled worldwide. Excessive intake of sugar is not good for a healthy lifestyle as it is devoid of nutritional benefits made of empty calories. Not only being dangerous for diabetics, it can also cause defect in pumping mechanism of the heart. Reduction of sugar intake in our diet helps us to keep the lifestyle diseases at bay and to maintain a healthy weight (Lustig *et al.,* 2012).

Majority of Indians fore choose vegetarian diets and prefer tea with sugar at least three times a day. Sugar is deliberately one of the major contributory in increasing diabetes in India (Mishra, 2011) which ranks second in the world for the production and consumption of sugar approximately of 16% of the world (Shukla *et al.,* 2017). Every country that has adopted the Western diet — one dominated by low-cost, highly processed food — has witnessed rising rates of obesity and related diseases. There are now 30% more people who are obese than who are undernourished (Lustig *et al.,* 2012).

Consumption of high quantities of fat and sugar, sugar sweetened snacks and beverages and leading a sedentary lifestyle is a major nutritional problem faced by mankind in this 21st century and is associated with serious health problems like diabetes and metabolic disorders like obesity (Stephen and Mark, 2011). Diabetes is a real threat to population worldwide, whose global prevalence has increased six times over the past twenty years (World Health Organization, 2016).

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with defects in insulin secretion, insulin action, or both resulting in disturbances of carbohydrate, fat and protein metabolism (World Health Organization, 1999). Diabetes currently affects 246 million people all over the world and is expected to reach 380 million by 2025. Every year, 3.8 million deaths are attributing to diabetes, representing over six percent of the total number of

deaths in the world. Some or the other die every ten seconds of diabetes-related causes and diabetes is the fourth leading cause of death by disease in the world (Chouhan *et al.*, 2016).

Diabetic patients are increasing day by day in India with more than 50 million with Type II Diabetes and according to world diabetes foundation, India has the world's largest diabetes population, followed by China with 43.2 million in 2000. India is labelled as 'Diabetic capital of world' and it has major concern among healthcare professionals and experts in national and international levels. It is predicted that the prevalence of diabetes doubles globally to 366 million in 2030 from 171 million in 2000 with maximum increase in India afflicting upto 79.4 million individuals in India (Wild *et al.,* 2004).

Diabetes being one of the expensive health problems, out of the total healthcare expenditure worldwide in 2010, healthcare expenditure on diabetes accounted for 11.6 per cent (Kumar *et al.*, 2012). One in eight individuals in India is diabetic. The average onset of diabetes in India is around 40 years while it is 55 years in other countries (Srilakshmi, 2014). In Tamil Nadu, one out of 10 people is diabetic, and every two persons in a group of 25 are in the pre-diabetic stage (https://timesofindia.indiatimes.com/city/chennai/1-in-10-people-in-Tamil-Nadu-is-diabetic/articleshow/7096511.cms). According to the first INDIAB Study supported by the Indian Council of Medical Research there are about 42 lakh individuals with diabetes and 30 lakh people with pre-diabetes in Tamil Nadu (http://www.thehindu.com/sci-tech/health/42-lakh-individuals-with-diabetes-in-TN-says-INDIAB-Study/article15591914.ece). Coimbatore district has around 2.8 lakh people with diabetes and 3.2 lakh people having pre-diabetes as quoted in an Indian Council of Medical Research - India Diabetes (ICMR-INDIAB) study (http://www.thehindu.com/news/cities/Coimbatore/coimbatore-district-has-28-lakh-diabetics-says-study/article2032749.ece).

The complications of diabetes mellitus include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). According to a study conducted by Paulraj *et al.,* (2017) 35% of patients with diabetes mellitus showed depression as symptom associated with unemployment and co-existent hypertension.

Many oral hypoglycemic agents and insulin treatments are available in Indian market such as biguanides and sulfonylureas and are used to reduce hyperglycemia for the treatment of diabetes mellitus, though the oral hypoglycemic drug therapy is not satisfactory due to their side effects, being a big challenge to the medical community (Mishra, 2011).

Various Indian traditional herbs like fenugreek, *Vinca rosea* and kurri patta are available and consumed by the Indians to control and prevent diabetes. Even though these traditional anti-diabetics control diabetes to some extent, poor availability and high cost is also a big constraint in India. Moreover the eating habits and taste of the Indian population are few constraint factors at consumption level as they found fenugreek as bitter. A variety of sugar substitutes like saccharin, sucralose and aspartame gained importance in reducing calorie intake but these can cause more health problems than they cure (Balaswamy *et al.*, 2014). Thus there is a need for natural non-caloric sweetener with acceptable taste and health properties.

Substitution of low calorie sweeteners in place of sugars helps in effective weight management and to prevent diabetes among people. *Stevia rebaudiana* native to Paraguay is a perennial shrub of the Asteraceae family, which has been introduced and cultivated all over the world as it can be grown under different climatic conditions; is a endemic and medicinal herb (Sivaram and Mugundan, 2003)

Stevia rebaudiana commonly known as sweet leaf or sugar leaf is an anti-diabetic sweetener herb and can sweeten a number of foods and beverages in the form of fresh or dried leaves or tincture. *Stevia rebaudiana* offers fewer calories showing no side effects after consumption. The leaves of *Stevia rebaudiana* exhibit an intensely sweet taste due to diterpenic glycosides such as stevioside and rebaudioside A that are approximately three hundred and four hundred times sweeter than saccharose, respectively (Santini *et al.*, 2008) which can be utilized as a substitute to sucrose (Robinson, 1930; Soejarto *et al.,* 1983; Lyakhoukin *et al.*, 1993; Matsui 1996; Megeji *et al.*, 2005; Sekaran *et al.*, 2007) to diet conscious consumers.

Stevia rebaudiana is gaining popularity in various developed and developing countries as an important crop for the production of non-nutritive, non-

toxic, high-potency sweeteners (Yadav *et al.*, 2011). *Stevia rebaudiana* has many other curative properties, such as the inhibition of bacterial and fungal growth, and it is an anti-cancerous, anti-hyperglycaemic, anti-hypersensitive agent, it prevents dental caries and has contraceptive properties (Yadav and Guleria, 2012). The leaves have many medical applications like antimicrobial, antiviral, antifungal, antihypertensive, anti-inflammatory, etc. The toxicological studies have shown that secondary metabolites present in *Stevia rebaudiana* does not have teratogenic, mutagenic or carcinogenic effect and no allergic reactions have been observed after consuming it as sweetener (Arora and Jood, 2016).

The leaves of *Stevia rebaudiana* contain protein, fat, carbohydrates, vitamins like folic acid, vitamin C and minerals including potassium, calcium, magnesium, phosphorus and all indispensable aminoacids except tryptophan. Some of the flavonoid polyphenolic anti-oxidant phytochemicals are kaempferol, quercetin, chlorogenic acid, caffeic acid, isoquercitrin, iso-steviol, etc. It is used in traditional medicine by native South Americans to help treat skin disorders, prevention of tooth decay and as a tonic to treat depression. Other benefits include improved energy levels, strengthening immune system, stimulating mental activity, and may also help in withdrawal from tobacco and alcohol addiction (Rieck, 2012).

Leaves of *Stevia rebaudiana* being a part of ancient Ayurvedic system (Megeji *et al.*, 2005) used for years as a sweet alternative to sucrose (Goyal *et al.,* 2010). It can normalize blood pressure levels, to regulate heartbeat and its hot water extract has shown to lower both systolic and diastolic blood pressure in human useful as a heart tonic beneficial to human health. It acts at the cell membrane level in the same way that calcium channel blocking agent by relaxing the muscular walls of the arteries causing elevation in blood pressure to help control high blood pressure by (Gardana *et al.*, 2010).

Stevia rebaudiana leaves have stevioside content of 10% to 12% on dry basis, yielding sweetness that is non-fermenting and does not exhibit browning on cooking that even 50 g of *Stevia rebaudiana* leaves can replace 1000 g of cane sugar (Mishra, 2011). Sweet and fatty food craving can be reduced by the consumption of *Stevia rebaudiana* leaves (Jain *et al.,* 2007). It can be used as a nutritive sucrose substitute beneficial to fight against dental caries. Having potent

sweetness intensities and providing a cost effective sucrose substitute, *Stevia rebaudiana* leaves can change a person's health risk by a slight alteration in the diet alternative to sucrose (Gupta *et al.,* 2013). *Stevia rebaudiana* has microbicidal property that can inhibit the growth of certain bacteria and infectious microbes helping to prevent the onset of colds and flu. It is used in traditional way of treating wounds, sore and gum disease (Ghosh *et al.,* 2008). It is advocated for those who are susceptible to yeast infections or reoccurring streptococcal infections which is commonly aggravated by consumption of white sugar (Debnath, 2008).

Chlorogenic acids present in *Stevia rebaudiana* leaves reduce conversion of glycogen to glucose and reduce absorption of glucose and thereby reduce the blood sugar level. It is used as a flavour enhancer, taste enhancer and anti-bacterial effect and heat stable at high temperatures that can be cooked along food. It can be used widely in jams, sauces, jelly, confections, beverages, pharmaceutical, alcoholic beverages and in dental products (Mishra, 2011). People worldwide suffer from dental caries which affects them throughout their lifetime. This is one of the most dominant chronic diseases of the oral cavity that destroys the tooth structure. *Stevia rebaudiana* leaves contain bacteriostatic and bacteriocidal properties that abolish the cause of dental caries and gingivitis (Gupta *et al.,* 2013). The increasing demand of consumers for herbal foods may encourage *Stevia rebaudiana* cultivation and production and would help to enjoy the sweet taste with minimal calories for those who have to restrict carbohydrate or sugar in their diet (Ranjan *et al*., 2011).

Only very few Indian studies are available with *Stevia rebaudiana* leaves in developing diabetic friendly products and keeping this in mind, an attempt was made in the present study, with the following objectives to:

- determine the proximate principles in *Stevia rebaudiana* leaves
- analyze the phytochemical constituents, antimicrobial activity and toxins present in the *Stevia rebaudiana* leaves
- develop diabetic friendly *Stevia rebaudiana* substituted beverages and desserts
- determine the glycemic index of the developed desserts substituted with *Stevia rebaudiana* leaves

II. REVIEW OF LITERATURE

The review literature pertaining to the study "**Evaluation and development of beverages and desserts substituted with *Stevia rebaudiana* leaves**" is presented under the following headings:

A. *Stevia rebaudiana* – a sweet herb
B. Nutritional and therapeutic importance of *Stevia rebaudiana* leaves
C. *Stevia rebaudiana* leaves as a sweetener
D. Role of *Stevia rebaudiana* in food products

A. *Stevia rebaudiana* – a sweet herb

Stevia rebaudiana is a small perennial of Asteraceae family (Gupta *et al.*, 2013 and Kujur *et al.*, 2010) a sweet herb of Paraguay, growing up to 65-80 cm tall, with sessile, oppositely arranged leaves (Goyal *et al.,* 2010). It is self-incompatible plant and the pollination behaviour is entomophilous (Yadav *et al.,* 2011). Among 154 members of genus *Stevia* containing potential sweetening compounds, *Stevia rebaudiana* is the sweetest of the all. It grows easily in semi-humid subtropical climate in soil with a pH range 6.5-7.5. The leaves are the most valuable part of the *Stevia* plant since it contains most of the sweetening compounds like steviosides and other nutrients (Samsudin and Aziz, 2013). *Stevia rebaudiana* has a great potential as a new agricultural crop since consumer demand for herbal foods are increasing. *Stevia* cultivation and production would further help those who have to restrict carbohydrate intake in their diet to enjoy the sweet taste with minimal calories (Lemus-Mondaca *et al.*, 2012). *Stevia rebaudiana* is a plant that offers sweetness with fewer calories and do not show any side effects after consumption on human health; has many pharmacological and therapeutic applications; non-toxic and possess antioxidant, antimicrobial, antifungal and anticarcinogenic activity. *Stevia* is likely to become a major source of high potency low calorie sweetener for growing natural food market in future (Gupta *et al.*, 2013).

Stevia rebaudiana leaves are the useful part of the plant containing more than 30 different steviol glycosides (Ramos-Tovar and Muriel, 2017) that produce a sweet taste without caloric value. This herbal sweetener has been used by native Guarani Indians for centuries, to counteract the bitter taste of various plant-based

medicines and beverages. Due to its adaptability to wide climatic range, the high-sweet content, and its significant contribution to the welfare of human life it is of great value (Ramesh *et al*., 2006). Being an introduced crop in India (Kaushik *et al.,* 2010) and due to the increasing demands for the natural sweeteners, farmers of India are driven towards large-scale cultivation of *Stevia* (Goyal *et al.,* 2010). *Stevia rebaudiana*, an outstanding herb bearing leaves of very refreshing sweet taste and remarkable health promoting activities is rich in nutrients, containing substantial amounts of protein, calcium, phosphorous, sodium, magnesium, zinc, rutin, vitamin A, vitamin C and other nutrients, yet has no caloric value. Phytochemical screening of the crude extracts revealed the presence of secondary compounds such as alkaloids, flavanoids, steroids, tannins and phenols (Preethi *et al*., 2011).

Stevia rebaudiana Bertoni contains a complex mixture of labdane, diterpenes, (Lemus-Mondaca *et al*., 2012) triterpenes, stigmasterol, tannins, volatile oils, and eight diterpenenic glycosides: stevioside, steviobioside, dulcoside, and rebaudiosides A, B, C, D, and E ranging between 30 and 320 times sweeter than sugar (Brandle *et al*., 1998, Kujur *et al.,* 2010 and Lemus-Mondaca *et al*., 2012). The most abundant substances are stevioside and rebaudioside A. Of the *Stevia* glycosides, rebaudioside A is the sweetest and the most stable especially thermostable even at temperatures of upto 200 °C making it suitable in cooked foods (Lemus-Mondaca *et al*., 2012), and it is less bitter than stevioside. Rebaudioside E is as sweet as stevioside, and rebaudioside D is as sweet as rebaudioside A, while the other glycosides are less sweet than stevioside. Other chemicals with no sweet taste are also found in *Stevia* species and some may even be bitter in taste (Goyal *et al.,* 2010).

Rebaudioside A, as an individual steviol glycoside, is of particular interest in global sweetener market due to its most desirable and superior flavour profile as compared to stevioside having after taste bitterness (Yucesan *et al.,* 2016). Steviol glycosides, have recently been approved in western countries as sources of intense natural sweeteners (Kafle *et al.,* 2017). The use of steviol glycosides as non-caloric sweeteners has proven to be beneficial for patients with Type 2 Diabetes Mellitus (T2D), obesity and metabolic syndrome (Panagiotou *et al*., 2018).

Stevia rebaudiana experimentally cultivated in the Volturno river plain (Caserta, Italy) considering three diverse irrigation levels (T0, T50 and T100 – 0, 50 and 100% of restitution of soil water) and four harvesting times highlighted that SGs were produced similarly under both irrigation and drought stress conditions, but irrigation management exerted a greater influence on dry leaf matter productivity (Pacifico *et al.,* 2017). Stimulation of chilling stress with a pre-treatment with endogenous signalling components and in particular with salicylic acid (SA), hydrogen peroxide (H_2O_2), 6-Benzyl Amino Purine (BAP) and calcium chloride ($CaCl_2$) had an influence on the production of secondary metabolites that could induce tolerance to chilling and could constitute a suitable way to maintain quality and quantity of steviol glycosides under controlled artificial environment (Soufi *et al*., 2016).

The effects of different concentrations (0, 0.1, 1.0, 10, 100 or 1000 mg L^{-1}) of engineered zinc oxide (ZnO) nanoparticles (34 nm in size) on growth parameters, steviol glycosides (rebaudioside A and stevioside) production and antioxidant activities in the tissue culture grown shoots of *Stevia rebaudiana Bertoni* was experimented. The highest percentage of shoot formation (89.6%) at 1 mg L^{-1} of ZnO nanoparticles concentration suggests a positive influence of ZnO nanoparticles on *Stevia rebaudiana* growth as compared to other treatments with or without ZnO nanoparticles (Javed *et al*., 2017). Incorporation of a range of higher concentrations of $CuSO_4$-$5H_2O$ in MS medium significantly enhanced direct shoot bud induction and proliferation from cultured leaf and nodal explants taken from mature plants of *Stevia rebaudiana Bertoni* (Kalpana *et al*., 2010).

Jitendra *et al*., (2012) highlighted the recent development and achievements made for the micropropagation of *Stevia rebaudiana Bertoni* (an antidiabetic sweetener herb) in Hadoti region of south-east Rajasthan where he noted that shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments was highest in MS medium supplemented with 0.5 mg/l BAP+2.0 mg/l Kn, highest rooting was recorded on MS medium with 1.0 mg/l IBA. Raina *et al*., (2013) devised strategies to produce fertile seeds of *Stevia rebaudiana*. A mixed population of compatible genotypes should be raised to obtain fertile seeds, wherein both the morphotypes investigated had only 22 chromosomes but differed on the basis of leaf shape,

shoot collar diameter, plant type (compact or loose) and stevioside or rebaudioside A content.

B. Nutritional and therapeutic importance of *Stevia rebaudiana* leaves

Medicinal uses of *Stevia rebaudiana* include regulating blood sugar, preventing hypertension, treatment of skin disorder, and prevention of tooth decay. It also possesses antibacterial and antiviral properties (Kujur *et al.*, 2010). Proximate analysis done in *Stevia rebaudiana* leaves, showed that the protein, fat, carbohydrate and ash content were found to be 20.42, 4.34, 35.20 and 13.12 g/100 g dry weight basis respectively (Tadhani and Subhash 2006).

The leaves were also found to contain 13 types of minerals including potassium (K), calcium (Ca), magnesium (Mg) and phosphorous (P) at 2.51, 1.55, 0.5 and 0.35 mg/100 g respectively (Samsudin and Aziz, 2013). *Stevia* also contains folic acid, vitamin C and all of the indispensable amino acids with the exception of tryptophan (Lemus-Mondaca *et al.*, 2012). Steviol glycosides (SGs) are the secondary metabolites responsible for the sweetness of *Stevia* which are synthesized by SG biosynthesis pathway operating in the leaves and out of various SGs, stevioside and rebaudioside A are the major metabolites that are non-mutagenic, non-toxic, antimicrobial, and do not show any remarkable side-effects upon consumption (Yadav and Guleria, 2012). Stevioside along with compounds like rebaudioside A, steviol and isosteviol offers therapeutic benefits, as they have anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory actions. It was proposed for its role as a drug modulator, as steviol can interact with drug transporters (Varanuj *et al.,* 2009).

Bioactive compounds in *Stevia rebaudiana* leaves such as the phenolics, in association with steviol glycosides exert anti-caries, chemopreventive, insulinotropic and diuretic properties (Ameer *et al.*, 2017) antioxidant, antimicrobial, antihypertensive, antidiabetic, antiobesity, antihyperlipidemic serving as a natural and alternative treatment for diseases that are associated with metabolic syndrome, thus contributing to health promotion (Areli *et al.*, 2017). Muanda *et al.*, (2011) evaluated the chemicals compounds, antioxidant, anti-inflammation and antimicrobial activities in essential oil (EO), water extract (WE), and methanol–water (MWE) (50/50 v:v) prepared from *Stevia rebaudiana Bertoni* leaves. The EO

was analyzed by gas chromatography/mass spectrometry, in which carvacrol, caryophyllene, caryophyllene oxide, spathulenol, cardinol, α-pinene, limonene, isopinocarveol and ibuprofen were identified as major compounds. The WE, and MWE compounds were identified by RP-HPLC, the major compounds were, quercetin dihydrate, protocatechuic acid and quercetin glucosyl that resulted in high antioxidant, anti-inflammation and antimicrobial properties of *S. rebaudiana* EO and extracts. The antimicrobial and antifungal activities of the extracts (EO, WE, MWE) were tested on *Staphylococcus aureus*; *Bacillus subtillis*; *Escherichia coli*; *Pseudomonas aeruginosa*; *Aspergillus niger* and *Candida albicans*, the lowest activity was founded on the EO extract.

Antimicrobial properties

Antimicrobial and antitumor activities of *Stevia rebaudiana* leaf extracts on four solvent extracts (ethyl acetate, acetone, chloroform and water) of *Stevia rebaudiana* leaves against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Bacillus subtilis, Aeromonas hydrophila* and *Vibrio cholerae* using agar well diffusion method showed that among the four extracts tested, acetone extract had effective antibacterial potential, followed by ethyl acetate extract. The acetone extract showed greater activity against gram-positive than against gram-negative organisms. *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Epidermophyton* species were used to test anti-yeast and antifungal activity in which, all the extracts were active against *Epidermophyton* species and *Candida albicans*. The 1:8 dilution of the acetone extract was non-toxic to normal cells and also had both anticancer and anti-proliferative activities against cancerous cells (Jayaraman *et al.*, 2008).

The antimicrobial potential of *Stevia rebaudiana* chemical extract from its leaves were subjected to microbial assay using six solvents against ten selected pathogenic as well as food spoiling fungal (*Alternaria solani, Helminthosporium solani, Aspergillus niger, Penicillium chrysogenum*) and pathogenic bacterial (*Escherichia coli, Bacillus subtilis, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*) isolates in which, 250µg/ml of petroleum ether extract (minimum inhibitory concentration) was found sufficient enough to inhibit the growth of test microorganism *E.coli* completely in petriplates

(by plate dilution method) which concluded that plant extracts of *Stevia rebaudiana* Bertoni leaves may have a role to be used as pharmaceuticals and/or preservatives (Ghosh *et al.,* 2008).

Six solvent extracts from leaf, three solvent extracts from flower of *Stevia rebaudiana* were assayed for *in vitro* antibacterial activity against pathogenic bacteria such as *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas fluorescence*, the zone of inhibition were compared with different standard antibiotics (Preethi *et al*., 2011). Acetone and ethanol extracts of *Stevia* leaf gave the highest zone of inhibition against *Streptococcus mutans*. Aqueous extract of this plant was not effective on *Streptococcus mutans* (Sichani *et al*., 2012). The plant extracts of *Stevia rebaudiana* Bertoni leaves have a role to be used as pharmaceuticals or preservatives. By evaluating the *in vitro* antimicrobial potential and phytochemical screening of the crude extracts of leaves of *Stevia rebaudiana* Bertoni, highest antifungal index [(12.13 ± 0.08) mm)] and lowest antifungal index [(9.13 ± 0.04) mm] as well as highest antibacterial index [(11.89 ± 0.07) mm] and lowest antibacterial index [(7.24 ± 0.03) mm] were obtained for extracts B, H, A and F, respectively (Siddique *et al*., 2016).

In a study where stevioside and steviol were tested for mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 and for chromosomal effects on cultured human lymphocytes, Stevioside was not mutagenic at concentrations up to 25 mg/plate, but showed direct mutagenicity to only TA98 at 50 mg/plate. Steviol did not exhibit mutagenicity in either TA98 or TA100, with or without metabolic activation. No significant chromosomal effect of stevioside and steviol was observed in cultured blood lymphocytes from healthy donors. Thus, Stevioside and steviol are neither mutagenic nor clastogenic *in vitro* at the limited doses (Suttajit *et al*., 1993). Tadhani and Subhash (2006) confirmed the possible antimicrobial potentiality of the leaf extract of *Stevia rebaudiana* in which, water extract of Stevia leaf showed activity against *B. subtilis* and *S. aureus* only. Methanol extract gave the highest zone of inhibition against *P. aeruginosa* whereas minimum zone of inhibition was found against *S. aureus* and yeast. *B. megaterium* and yeast were found to be highly susceptible towards ethyl acetate and hexane extracts, respectively whereas *A. niger* and *B. subtilis* were found to be least susceptible

against ethyl acetate and hexane extracts, respectively. Hexane extract showed the highest activity against yeast among the tested microorganisms.

Antioxidant properties

The well-known antioxidant and anti-inflammatory properties of *Stevia rebaudiana* makes it a medicinal herbal, mainly used to treat diabetes, an excellent therapeutic strategy to fight liver diseases and providing beneficial effects on liver damage (Ramos-Tovar and Muriel, 2017). Singh *et al.*, (2012) investigated the antioxidant activity of methanolic extracts from root, leaf, stem and flower of *Stevia rebaudiana* (Bertoni) and revealed it as an excellent antioxidant and anti-bacterial, especially root (64.23 ± 8.35 mM) and leaf (56.26 ± 16.87 mM) among which high amounts of phenols, flavonoids and tannins were found assessed by 2,2'-azino-bis-3-ethylbenzo-thi8azoline-6-sulfonic acid (ABTS) radical scavenging activity assay as well as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. *Stevia* extract improves the functional food properties due to its strong antioxidant properties. The effect of harvest time, experimental site and crop age on the no-calorie sweetener steviol glycosides and on the antioxidant properties of stevia leaf extracts were analyzed which defined a high level of phenols (78.24 mg GAE g^{-1} DW by Folin–Ciocalteu method) and high antioxidant activity (812.6 µmol Fe^{2+} g^{-1} DW by FRAP assay). The inhibition of DPPH free radicals was evaluated and an IC_{50} mean value of 250 µg mL^{-1} (Tavarini and Angelini, 2013).

At 0.1 mg/mL, the ethyl acetate extract (EAE) of the crude 85% methanolic extract (CAE) of *Stevia rebaudiana* leaves exhibited preventive activity against DNA strand scission by OH generated in Fenton's reaction on pBluescript II SK (–) DNA whose efficacy was better than that of quercetin. The total polyphenols and total flavonoids of EAE were 0.86 mg gallic acid equivalents/mg and 0.83 mg of quercetin equivalents/mg, respectively. Flavonoids, isolated from EAE characterized by LC-MS and NMR analysis, were quercetin-3-O-arabinoside, quercitrin, apigenin, apigenin-4-O-glucoside, luteolin, and kaempferol-3-O-rhamnoside (Ghanta *et al.*, 2007).

The DPPH activity of the ethanolic leaf extract (20, 40, 50, 100 and 200 µg/ml) of *Stevia rebaudiana* increased in a dose dependent manner, in the range of 36.93–68.76% as compared to ascorbic acid 64.26–82.58%. The IC_{50} values of

ethanolic extract and ascorbic acid in DPPH radical scavenging assay were obtained to be 93.46 and 26.75 µg/ml, respectively. It was found that the ethanolic extract scavenges the superoxide generated by EDTA/NBT system. The total phenolic content using Folin–Ciocalteau reagent was 61.50 mg/g significantly higher to reference standard gallic acid and the ethanolic extract also inhibited the hydroxyl radical, nitric oxide, superoxide anions with IC_{50} values of 93.46, 132.05 and 81.08 µg/ml respectively (Shukla *et al.*, 2009).

As a source of edible plant-based antioxidant *Stevia rebaudiana* stem waste investigation with hot water extract revealed that it had significantly higher antioxidant activity against fish oil oxidation than that of the leaf, despite having lower total phenolic content, DPPH radical scavenging activity and ORAC values (Yu *et al.*, 2017). Arriola *et al.*, (2016) encapsulated aqueous leaf extract of *Stevia rebaudiana* Bertoni with sodium alginate and evaluated the effect on the total phenolic content (TPC) and antioxidant stability. High encapsulation efficiency values were obtained for the wet (69.8%) and for the lyophilised (97.7%) beads stored in the optimised extract lyophilisation noticeably affected bead size and morphology, and turned out to be an appropriate method for preservation of encapsulated polyphenols. Encapsulation of *Stevia* extracts in alginate beads is an inspiring technique for food supplementation with natural antioxidants.

Anti-hypertensive effect

Chan *et al.*, (2000) revealed that oral stevioside is an effective and well tolerated modality acting as an alternative or supplementary therapy for patients with hypertension. Hypertensive participants with diastolic blood pressure between 95 and 110 mm Hg and ages ranging from 28 to 75 years with 60 subjects were allocated to active treatment by capsules containing stevioside (250 mg) thrice daily and followed-up at monthly intervals for 1 year which after 3 months, the systolic and diastolic blood pressure of the participants decreased significantly and the effect persisted during the whole year.

Ulbricht *et al.*, (2010) evaluated two long-term studies which indicated that *Stevia* may be effective in lowering blood pressure in hypertensive patients, although data from shorter studies (1-3 months) did not support these findings. A pair of other studies also report positive results with respect to glucose tolerance

and response, although the relatively low methodological rigor of these experiments limits the strength of these findings. The hemodynamic effects of 4 weeks consumption of 1000 mg/day rebaudioside A in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) was evaluated which showed that rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-h ambulatory blood pressure responses (Maki *et al.*, 2008).

Hepatoprotective effect

Latha *et al.*, (2017) investigated the hepatoprotective effect of hydroalcoholic extract of *Stevia rebaudiana* leaves and its major phytochemical constituent, stevioside in LPS induced acute liver injury. The results revealed that both *Stevia rebaudiana* leaf extract and stevioside treatment ameliorated LPS induced hepatic oxidative stress, evident from altered levels of reduced SOD, catalase, GSH, MDA, NO. Histopathological observations revealed that both *Stevia rebaudiana* leaf extract and stevioside attenuated LPS induced structural changes and hepatocellular apoptosis providing additional evidence for its hepatoprotective effect.

Anti-diabetic effect

The *Stevia* leaf powder has been reported to reduce the blood glucose concentration of diabetic rats at a rate of 250 mg/kg body weight showing very potent hypoglycemic efficacy (Kujur *et al.*, 2010) due to steviosides counteracting the glucotoxicity in β-cells or also by suppressing the glucagon secretion by α-cell of pancreas (Chen *et* al., 2005 and Kujur *et al.*, 2010). *Stevia rebaudiana* leaf extracts have been used in traditional medicine in Brazil and Paraguay, in the treatment of diabetes (Jeppesen *et al.*, 2000) due to its numerous therapeutic properties, proven safe and effective over centuries (Shivanna *et al.*, 2013). Stevioside exerts anti-hyperglycaemic, insulinotropic, and glucagonostatic actions in the Type 2 Diabetic Goto-Kakizaki rats, having the potential of becoming a new anti-diabetic drug for use in Type 2 Diabetes. A direct insulinotropic effect in isolated mouse islets and the clonal beta cell line INS-1 of the glycoside stevioside in *Stevia rebaudiana* leaves was demonstrated wherein stevioside significantly suppressed the glucose response to the intravenous glucose tolerance test in

Goto-Kakizaki rats and concomitantly increased the insulin response though the glucagon level was suppressed (Jeppesen *et al.*, 2000).

Stevia rebaudiana whole leaves powder and extracted polyphenols when fed to rats resulted in a reduction of blood glucose, ALT, AST and MDA concentration in liver, with increment of insulin level and improved glucose tolerance, insulin sensitivity and antioxidant status through antioxidant enzymes (Shivanna *et al.,* 2013). The effect of *Stevia rebaudiana* leaf extract on diabetic patient of different age group and gender was found in which, *Stevia* leaf powder was taken under normal diet and comparison has been made with medicine, without medicine and with *Stevia* intake. *Stevia* powder was replaced with sugar and consumed with tea three times a day; significant decrease in FBS and PPBS was observed (Mishra, 2011). Mohd-Radzman *et al.*, (2013) studied the mechanisms involving free fatty acids, adipocytokines such as TNFα and PPARγ and serine kinases like JNK and IKKβ, asserted to be responsible in the development of insulin resistance in which the underlying constituents of medicinal significance found in the *Stevia rebaudiana* Bertoni plant (among other plants that potentiate anti-hyperglycemic activities) is crucial for comprehending the progression of insulin resistance towards the development of diabetes mellitus.

Sharma *et al.*, (2012) demonstrated the free-radical scavenging effects of *Stevia rebaudiana* bertoni standardized extract on diabetes-induced oxidative stress animal model. They aimed to investigate the effect of this extract on hyperglycemia and hepatic antioxidant enzymes of animal models of Type 2, Non-Insulin Dependent Diabetes Mellitus (NIDDM) and at the end of the experiment, hepatic antioxidant enzyme assays and lipid peroxidation clearly indicated that *Stevia* extract have significant antioxidant effect on diabetes pathology and also the administration of the extract reduced the abnormal blood sugar levels significantly ($p< 0.001$) and improved the hyperglycemic condition.

Cytoprotective effect

The sweet steviol glycosides have functional and sensory properties superior to those of many other high potency sweeteners. The PESR, EESR & AESR has investigated the anti-ulcerogenic activity by using Wister rats in both

sexes. Oral administration of petroleum ether, ethanol & aqueous extracts of *Stevia rebaudiana* (100 & 300 mg, p.o) produced a significant ($p < 0.01$) and dose dependent inhibition to the acute ulcer induced by ethanol 99% of 2.5 ml/kg/body weight at once to rats and the parameters of gastric secretion were evaluated. The inhibitory effect of the *Stevia rebaudiana* on lesions induced by stress was compared to that of cimetidine, which concludes that the *Stevia rebaudiana* having the potential effectiveness at the dose of 300 mg/kg/body weight, <p 0.01 by dose dependent manner (Pandiyan *et al.*, 2009). The compound labdane sclareol, present in leaf extract of *Stevia rebaudiana* has anti-tumorous and cytotoxic properties (Kaushik *et al.*, 2010).

Toxicity studies

Acute toxicity studies conducted by Kujur *et al.*, (2010) revealed that the administration of graded doses of three crude aqueous, ether, and methanol extracts (up to a dose of 5000 mg/kg) of *Stevia rebaudiana* did not produce noticeable changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma, and appearance of the animals when no death occurred, all mice being physically active. Zhang *et al.*, (2017) evaluated the toxicity of ethanolic extract of *Stevia rebaudiana* leaves through a battery of *in vitro* and *in vivo* tests where, oral administration at dietary levels of 1.04%, 2.08% and 3.12% for 90 days did not induce significant behavioral, hematological, clinical, or histopathological changes in rats. Significant reduction of cholesterol, total protein and albumin was observed in female animals only at high dose level. The results demonstrated that ethanolic extract of *Stevia rebaudiana* leaves rich in isochlorogenic acids, does not possess adverse effects through oral administration.

C. *Stevia rebaudiana* leaves as a sweetener

Stevioside and rebaudioside A are the main sweetening compounds that are thermostable even at temperatures of up to 200 °C, making them suitable for use in cooked foods (Lemus-Mondaca *et al.*, 2012). Standard extracts of *Stevia rebaudiana* are used as natural sweetener or dietary supplements in different countries for their content of stevioside or rebaudioside A. These compounds possess upto 250 times the sweetness intensity of sucrose and are non-calorigenic (Kujur *et al.*, 2010). The plants leaves, the aqueous extract of leaves, and purified

steviosides are used as sweeteners (Midmore and Rank 2002, Kujur *et al.*, 2010). Leaves of Paraguan *Stevia* contain 9 – 13% of the sweet steviosides/rebaudiosides molecules, Chinese Stevia contains only 5 – 6% and Indian *Stevia* contains about 9.08% steviosides based on dry weight of leaves (Yadav *et al.*, 2011).

Freshly harvested *Stevia rebaudiana* leaves contain about 80% moisture content and will deteriorate easily if not properly dried. *Stevia* leaves have to be dried within 8 hour after harvest in order to retain the high level of sweetness (Samsudin and Aziz, 2013). The high-purity *Stevia rebaudiana* leaf extract, is being used globally to reduce energy and added sugar content in food and beverages thereby act as a new tool to help achieve weight management goals (Ashwell, 2015). The hot water crude extract of *Stevia rebaudiana* leaves is 20 times sweetener than sugar with full of good minerals when used as a sweetener in different foods and drinks (Abdo and Genet, 2016). Serial dilution of the hot water crude extract of *Stevia rebaudiana* leaves and aqueous solution of table sugar were subjected to choose the preferable sweetening level from both solutions independently from which, the *Stevia* and sugar ratio were corresponded for individual panelist perception. 2.5 mg/mL *Stevia* solution and 50 mg/mL sugar solution for sweetening was preferred. This ratio shows 1 g of *Stevia* is equivalent with 20 g of sugar (Abdo and Genet, 2016).

Stevioside analysis based on the water extraction, hydrophobic chromatography (Sep-Pak C18cartridges) and HPLC using linear gradient of acetonitrile in water described by analyzing the stevioside level in *Stevia* leaves as well as in tea proved to be fast and friendly to the environment by minimization of organic solvent consumption for stevioside analysis (Vanek *et al.,* 2001). The most abundant sugars were xylose, arabinose with fructose and sucrose, presenting dried samples with higher contents than frozen fresh ones, while the latter better retained tocopherols than dry samples (Barroso *et al.*, 2018). Yadav *et al.*, (2011) described that Stevioside and rebaudioside-A are negatively correlated, while rebaudioside-A and rebaudioside-C are positively correlated. Conventional plant breeding approaches such as selection and intercrossing among various desirable genotypes is the best method for improving quality traits in a highly cross-pollinated crop like *Stevia*. Huang *et al.*, (2010) successfully isolated and purified three steviol glycosides, stevioside, rebaudioside A and rebaudioside C from the extract of

leaves of *Stevia rebaudiana* Bertoni by High-Speed Counter-Current Chromatography (HSCCC). In a single operation, 200 mg of the crude extract yielded pure stevioside (54 mg), rebaudioside A (36 mg), and rebaudioside C (13 mg) with the purities of 98.3%, 98.5% and 97.6%, respectively.

Adari *et al.*, (2016) developed a novel insitu enzymatic transglycosylation of stevioside by pre-treating the *Stevia* leaves with cellulase and adding soluble starch as the glucosyl donor and confirmed the transglycosylation of stevioside led to an enrichment in the rebaudioside-A content from 4% to 66%. This study highlighted the biotransformation of stevioside present in *Stevia* leaves to rebaudioside-A by a simple, inexpensive and eco-friendly process that has commercial potential. Langle *et al.*, (2015) in a major finding of his study stated that the TiO_2-*Stevia rebaudiana* (20 and 30 µM) has a potent and prolonged activity to be anti-diabetic. Chronic administrations of *Stevia rebaudiana* are required to cause the normoglycemic effect. TiO_2 nanomaterials with *Stevia rebaudiana* at different concentrations (10, 20 and 30 µM) by sol–gel method observed as hemispherical agglomerated particles of different sizes. The nanomaterials were evaluated in male rats whose diabetes mellitus-phenotype was induced by alloxan (200 mg/kg, i.p.). The co-administration of TiO_2-SrB (20 and 30 µM) induced a significant and permanent decrease in the glucose concentration since 4 hours, until 30 days post-administration.

D. Role of *Stevia rebaudiana* in food products

Stevia is likely to become a major source of high potency low calorie sweetener for growing natural food market in future (Gupta *et al.,* 2013). *Stevia rebaudiana* is a nutrient rich natural sweetest plant of Asteraceae family. The leaves naturally contain diterpene glycosides stevioside, rebaudiosides A-F, steviolbioside and dulcoside, which are responsible for its sweet taste and have commercial value all over the world as sugar substitute in foods, beverages or medicines. It is a plant which offers sweetness with fewer calories and do not show any side effects after consumption on human health (Gupta *et al.*, 2013). Anton *et al.*, (2010) tested the effect of preloads containing *Stevia*, aspartame, or sucrose on food intake, satiety, and postprandial glucose and insulin levels in which the preload order was balanced, and food intake (kcal) was directly calculated. Hunger

and satiety levels were reported before and after meals, and every hour throughout the afternoon. Participants provided blood samples immediately before and 20 minutes after the lunch preload. Despite the caloric difference in preloads (290 *vs.* 493 kcals), participants did not compensate by eating more at their lunch and dinner when they consumed *Stevia* and aspartame versus sucrose in preloads.

Shah *et al.*, (2010) examined sucrose-free milk chocolates sweetened with *Stevia* and containing different types of commercial inulin or polydextrose as bulking agents in relation to their physicochemical, rheological and sensory properties. Compared with chocolate sweetened with sucrose, noticeable differences in lightness (L* values) were observed for sucrose-free chocolates and attributed to changes in surface roughness. Chocolate containing inulin with a higher degree of polymerisation (DP) had higher melting points, greater plastic viscosity and an increased flow behaviour index. Chocolate containing the highest DP inulin was found to be very similar to control in tested sensory attributes (appearance, firmness, smoothness, mouth feel, flavour/taste and overall acceptance) when assessed by a consumer panel. The data indicates that it is possible to manufacture sucrose-free chocolate using high DP inulin without adversely affecting its important physicochemical properties and sensory acceptance. Oliveira *et al.*, (2012) in a study described new process denominated dual stage sugar substitution (D3S) which aims to substitute high calorie sugars of Malay apples for a low calorie natural sweetener. In a first stage, high calorie sugars (sucrose, fructose and glucose) were partially removed from the fruit samples and in a second stage, low calorie sugars (stevioside and rebaudioside) were incorporated to the fruit to maintain its sweetness. The use of ultrasound was evaluated on both stages of the D3S process. Best performance of the process was obtained by subjecting the fruit samples to ultrasound in the sugar removal stage followed by immersion of the samples in *Stevia*-based solution with application of ultrasound in the sweetener incorporation stage. These operating conditions resulted in the highest sugar removal during the first stage, highest water loss during the process and highest sweetener incorporation during the second stage of the D3S process.

Capella *et al.*, (2017) processed a fruit juice-*Stevia* beverage using Pulsed Electric Fields (PEF), a non-thermal preservation technology, with the purpose of

investigating the feasibility of PEF for bioactive compounds and steviol glycoside enhancement and its impact on physicochemical properties. Variable ranges of response surface methodology were 20–40 kV/cm (electric field strength), 100–360 µs (treatment time) and 0–2.5% (w/v) *Stevia*. After PEF, ascorbic acid was retained by more than 74%. Some of the analyzed PEF treatments resulted in an enhancement of total anthocyanins and carotenoids. The best results for rebaudioside A/stevioside ratio were obtained when PEF was applied at 30 kV/cm for 230 µs. Hydroxy methyl furfural content and total color differences were maximum at the highest electric field strength assayed (40 kV/cm). PEF conducted at 21 kV/cm during 360 µs with 2.5% *Stevia* led to the beverage with the greatest content in bioactive compounds and sweetening properties with minimal color changes.

A negative off-flavour was found for the yoghurts with *Stevia* which was partially masked by actilight. The variant with 6% actilight together with *Stevia* had the closest profile to the variant with 8% sucrose. Sugar (8%) replacement with *Stevia*, with a combination of actilight (2–6%) and *Stevia* or with palatinose (8%) in set yoghurt was investigated. Skimmed milk with sucrose or sweetener was standardised to 4% or 5% protein, depending on the fat content (0.1% and 3.5%), then homogenised, heated to 92 °C and fermented at 42 °C until a pH of 4.6 was attained. The addition of sweeteners did not have a negative effect on the yoghurt making process or pH development (Guggisberg *et al.*, 2011).

Zahn *et al.*, (2013) evaluated steviol glycosides for partial replacement of sucrose in bakery products, muffins where 30% sucrose of the formulation was exchanged against an iso-sweet amount of rebaudioside A in combination with several fibres. Baked products were subjected to chemical, colour and texture analysis, and sensory characteristics were assessed by flash profiling. Multivariate analysis of instrumental and sensory data indicated that a combination of inulin or polydextrose with rebaudioside A resulted in products with characteristics close to those of a reference. The incorporation of these replacers reduced energy by 6 or 5 kJ/100 kJ, and increased fibre content from 1.3 g/100 g to 4.6 or 7.1 g/100 g, respectively. The use of wheat bran or apple fibre as bulk replacer for sucrose gave products which mainly deviated in crumb colour and are characterised by a

wholemeal off-taste, whereas increased crumbiness and reduced elasticity is the consequence of partial sucrose replacement by oat, pea or wheat fibre, cellulose or maltodextrin.

Hracek *et al.*, (2010) highlighted the use of steviosides in aqueous model systems resembling low-calorie sweet products useful to protect potassium sorbate (KS) from destruction and to decrease browning development. The steviosides promoted a slight increase in the Minimum Inhibitory Concentration (MIC) of sorbates against *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* in microbial stability. However, the main effect of steviosides was the protected action on sorbate destruction and was essential to ensure that the preservative residual level was higher than the MIC of the preservative to prevent the growth *Z. bailii* or *Z. rouxii* during storage.

Barba *et al.*, (2014) evaluated the optimal high pressure processing treatment (300–500 MPa, 5–15 min) combined with *Stevia rebaudiana* addition (0–2.5% (w/v)) to guarantee food safety while maintaining maximum retention of nutritional properties using response surface methodology. A treatment of 453 MPa for 5 min with a 2.5% (w/v) of *Stevia* succeeded in inactivating over 5 log cycles of *Listeria monocytogenes* and maximizing inactivation of PPO and POD, with the greatest retention of bioactive components.

A holistic view on consumers' hedonic food experience compared to what is traditionally seen in sensory research was studied by integrating the hedonic sensory experience and post-ingestive sensations to understand food satisfaction. Significant hedonic differences of sensory properties were found between apple-cherry fruit drinks with different levels of beta-glucan and different sweeteners, sucrose or *Stevia rebaudiana*, except between the fruit drinks varying in type of sweetener only. Satisfaction with sensory attributes was found to be the main drive of food satisfaction, while post-ingestive sensations drove satisfaction as well. While replacing sucrose with *Stevia rebaudiana* did not affect the hedonic and post-ingestive sensations, addition of beta-glucan resulted in both positive and negative post-ingestive sensations (Andersen *et al.*, 2017)

Complete purification of *Stevia* leaf extracts to obtain pure glycosides is not necessary for it to become a commercially acceptable sweetener. Variably

processed extracts enriched with polyphenols, pigments and a mixture of both were evaluated for sensory attributes by semi trained panel when added to coffee and lime juice. Presence of polyphenols influenced the acceptability of the sweeteners marginally, while chlorophyll was found unacceptable in any of the extracts. The antioxidant activity of the extracts was synergistic when it was mixed with coffee and lime juice (Kaushik *et al.*, 2010). Zulkifli *et al.*, (2016) demonstrated the incorporation of *Stevia* into cake formulations at different level (0%, 50%, 75% and 100%) that determined the effect of physicochemical properties of *Stevia* application in butter cake in place to sugar. Analytical testing on nutrients, calorie content, texture, color and percentage of mass sucrose carried out scientifically found that different level of *Stevia* content resulted in different calorie content.

Kroyer (2010) evaluated the stability of the natural sweetener stevioside during different processing and storage conditions as well as the effects of its interaction with water-soluble vitamins, food relevant organic acids and other common low calorie sweeteners and its application in coffee and tea beverages. Incubation of the solid sweetener stevioside at elevated temperatures for 1 hour showed good stability up to 120°C, whilst at temperatures exceeding 140°C forced decomposition was noticed. In aqueous solutions stevioside is remarkably stable in a pH range 2–10 under thermal treatment up to 80°C; however, under strong acidic conditions (pH 1) a significant decrease in the stevioside concentration was detected. Up to 4 h incubation of stevioside with individual water-soluble vitamins in aqueous solution at 80°C showed no significant changes with regard to stevioside and the B-vitamins, whereas a protective effect of stevioside on the degradation of ascorbic acid was observed resulting in a significant delayed degradation rate. Moreover, stevioside in solutions of organic acids showed a tendency towards enhanced decomposition of the sweetener at lower pH values depending on the acidic medium.

Jentzsch *et al.*, (2016) detected the counterfeit *Stevia* products using a versatile analytical technique, Raman spectroscopy which was capable of detecting contents as low as 5% (w/w) of sodium cyclamate during measurements of *Stevia*-sodium cyclamate mixtures. The findings show that Raman spectroscopy can successfully be used to detect counterfeit stevia and underline its high potential for the detection of food adulteration.

III. METHODOLOGY

The methodology followed for the present study entitled "**Evaluation and development of beverages and desserts substituted with *Stevia rebaudiana* leaves**" is presented as follows:

A. Procurement of *Stevia rebaudiana* leaves and analysis of proximate principles, phytochemicals, antimicrobial activity and toxic substances
B. Development and standardization of beverages and desserts substituted with *Stevia rebaudiana* leaves
C. Evaluation of organoleptic characteristics of *Stevia rebaudiana* leaves substituted beverages and desserts
D. Determination of glycemic index for the *Stevia rebaudiana* leaves substituted desserts
E. Statistical analysis and interpretation of the data

A. Procurement of *Stevia rebaudiana* leaves and analysis of proximate principles, phytochemicals, antimicrobial activity and toxic substances

Procurement and storage of *Stevia rebaudiana* leaves

Health is now becoming a major concern among the world population, such that more importance is given to natural foods which not only provide more nutrients, but also good taste (Tejo *et al.,* 2013). Most of the artificial sweeteners with a high degree of sweetness are produced from synthetic ingredients or exclusively synthesized by chemicals in the laboratory which are harmful for human health (Jae-Yong *et al.*, 2011).

Stevia rebaudiana is a natural sweetener plant (Choudhary *et al.*, 2014). The leaves of the plant was selected for the study as it had health promoting phytochemical constituents (Zlabur *et al.*, 2013) with remarkable sweetening potential that benefits diabetic patients, for those interested in decreasing calorie intake, lowers blood sugar (Goyal *et al.,* 2010) and for children without causing cavities (Tejo *et al.,* 2013).

The shade dried *Stevia rebaudiana* leaves were procured from Sanjivini Herbals, Salem. The company has steadily risen as a highly distinguished manufacturer, exporter and supplier of *Stevia rebaudiana* that offers the best *Stevia rebaudiana* at the best price to the world with timely delivery. The leaves were procured in dried form in a bulk quantity of 1 kg.

The dried *Stevia rebaudiana* leaves were finely powdered using a mixer grinder and sieved using microsieve (0.8 mm) which had an olive green colour. To restore the quality and aroma, the powdered leaves were stored in airtight container at a cool and dry place. The samples were taken for analysis whenever required. *Stevia* rebaudiana leaves are exhibited in Plate 1.

Analysis of proximate principles of the *Stevia rebaudiana* leaves

The proximate principles of the dried *Stevia rebaudiana* leaves were analyzed in the Nutrition Laboratory of the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore following the standard procedures. They include moisture, protein, ash, fat, crude fibre, total carbohydrate, starch, energy, ascorbic acid, beta-carotene, calcium, iron and phosphorus. The analyzed proximate principles are shown in Plate 2.

Moisture

Estimation of moisture is one of the most often performed determinations in food analysis. Moisture is lost when food is heated not much higher than the temperature of boiling water or by allowing standing overnight over dehydrating agent or by heating over vacuum (AOAC, 1990).

Protein

The given sample is digested with concentrated sulphuric acid in a macrokjeldahl flask when nitrogen gets converted to ammonium sulphate. Ammonia is liberated by the action of strong alkali in a macrokjeldahl steam distillation apparatus. This nitrogenous substance is converted to ammonium borate by absorbing 2% boric acid and is titrated against N/70 sulphuric acid. The volume of acid required to bring the test sample to the

colour of the blank gives the acid equivalent to the ammonia from which protein is estimated (AOAC, 1990).

Ash

By continuous heating, the substance gets charred which can be used for the determination of minerals present (AOAC, 1990).

Fat

Ether extraction of the crude fat in vegetable products is carried out in a continuous extractor that is an apparatus in which the ether, after dissolving a portion of the fat of the material and discharging into the extraction flask, is volatized, condensed and again allowed to act on the material. The steps in the process are repeated continuously and automatically until the extraction is complete.

The soxhlet extraction used depends on the intermittent action of a glass syphon. The ether gradually condenses into the extraction tube containing the material until it rises to the top when it is discharged into the extraction flask (AOAC, 1990).

Crude fibre

Crude fibre, the organic residue consisting largely of cellulose, that is left after other carbohydrates and proteins have been removed by successive treatment with boiling acids and alkalies was found out (AOAC, 1990).

Total carbohydrate

In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490nm (www.biocyclopedia.com).

Total energy (Calorific value)

Energy was determined according to the method described by Sukkar (1985) using the Atwater factor. By this determination, 1 g of carbohydrate provides 4 kcal; 1 g of protein provides 4 kcal and 1 g fat provides 9 kcal.

Starch

The sample is treated with 80% alcohol to remove sugar and then starch is extracted with perchloric acid. In hot acidic medium, starch is hydrolysed to glucose and dehydrated to hydroxyl methyl perforate. This compound forms a green colour product with anthrone (AOAC, 1990).

Vitamin C

Vitamin C is a good reducing agent and it reduces the dye 2,6 dichlorophenol indophenol. In this reaction, the ascorbic acid itself is oxidisied to dehydro ascorbic acid. In the absence of interfering substances, the capacity of an extract of the sample to reduce a standard solution of the dye as determined by titration is directly proportional to the vitamin C content. Oxalic acid is not only used to reduce the pH of the extracting medium, thereby establishing vitamin C but also form complexes with metals like copper thereby preventing the catalytic oxidation of vitamin (AOAC, 1990).

Beta carotene

Carotene present is extracted with petroleum ether and the intensity of the color of the extract is compared with that of the standard solution using a colorimeter (AOAC, 1990).

Calcium

Calcium is determined by precipitating it as calcium oxalate and titrating the oxalate solution in dilute sulphuric acid against standard potassium permanganate (AOAC, 1990).

Iron

The food sample is oxidized with ignition or oxidation. Iron as ferric iron reacts with ammonium thiocyanate or with potassium thiocyanate to give ferric thiocyanate which is red in colour. The colour which is a measure of the concentration is measured colorimetrically (AOAC, 1990).

Stevia rebaudiana leaves

Storage of powdered and sieved leaves in airtight container

Plate 1

***Stevia rebaudiana* leaves**

Moisture

Ashing

Nitrogen estimation

Fat estimation

Plate 2

Analysis of proximate principles in *Stevia rebaudiana* leaves

Plate 2 (Contd…)

Total carbohydrate estimation

Vitamin C estimation

Beta carotene estimation

Starch estimation

Calcium estimation

Fibre estimation

Nutrient analysis of *Stevia rebaudiana* leaves

Phosphorus

When the ash solution is treated with ammonium molybdate, phosphomolybdic acid is formed. Phosphomolybdic acid is reduced by the addition of 1,2,4 Amino Naphthol Sulphonic Acid reagent to produce a blue colour which is apparently a mixture of oxides of molybdenum. The intensity of the colour developed is the measure of phosphorus present (AOAC, 1990).

Phytochemical analysis of *Stevia rebaudiana*

Phytochemicals are bioactive constituents that sustain or promote health which are broadly described as phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids and anthocyanidins. The health benefits of phytochemicals are prevention and or treatment of diseases and physiological disorders, either alone and or in combination, provide therapeutic potential and act as anti-inflammatory, anti-allergic, antioxidants, antibacterial, antifungal, antispasmodic, chemopreventive, hepato-protective, hypolipidemic, neuroprotective, hypotensive, anti-aging, hypoglycemic, prevent osteoporosis, DNA damage, cancer and heart diseases, induce apoptosis, CNS stimulant, analgesic, protects from UVB-induced carcinogenesis, immuno-modulator and carminative (Prakash *et al.,* 2012). The phytochemical analysis is exhibited in Plate 3.

Preparation of phytochemical extracts

a. Aqueous extract

The sample was used to prepare an infusion in hot (95°C) distilled water. The infusion was left overnight under refrigeration at 4°C to prevent any possible contamination. After 24 hours the extract was kept in rotary shaker at 100 rpm for an hour and filtered with Whatman No. 1 filter paper (Doughari *et al.*, 2012).

b. Solvent extracts

The sample was mixed with sufficient quantity of solvents viz., methanol and ethanol. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No. 1 filter paper (Doughari *et al.*, 2012).

The phytochemicals present in the sample was thus analyzed in both aqueous extract and in solvent extract using both ethanol and methanol as solvents.

Tannins- About 0.5 g of the dried power was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and was observed for brownish green or a blue black coloration (AOAC, 2005).

Terpenoids- To 0.5 ml of the extract, 2ml of the chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids (AOAC, 2005).

Phenols- To 1ml of the plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green indicates presence of phenols (AOAC, 2005).

Saponins (Foam test)- Five millilitre sample extract was dissolved in 2.5ml of dilute water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously and then emulsion was observed (AOAC, 2005).

Quinones- To 1ml of the extract, 1ml of the concentrated sulphuric acid was added. Red colour formation indicates the presence of quinone compound (AOAC, 2005).

Glycosides- To 2ml of the extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates the presence of glycosides (AOAC, 2005).

Coumarins- To 2 ml of the test solution, a few drops of alcoholic sodium hydroxide were added. Appearance of yellow colour indicates the presence of coumarin (AOAC, 2005).

Sterols (**Sulphuric acid Test)** - To the plant extracts 2 ml of chloroform was added. 2 ml of concentrated sulphuric acid was added by the sides of the test tube and observed for red colour at the lower chloroform layer (Shah and Shah, 2015).

Flavonoids (Shindo's test)- To the test solution, a few magnesium turnings and a few drops of concentrated hydrochloric acid was added and boiled for five minutes. Appearance of red or orange red colour indicates the presence of flavonoids (AOAC, 2005).

Alkaloids- One milliliter of aqueous extract was stirred and placed in 1% aqueous hydrochloric acid on a stream bath. Then, 1 ml of the filtrate was treated with Dragendorff s and Mayer's reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids (AOAC, 2005).

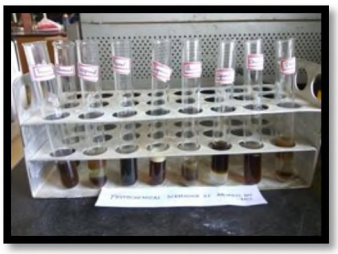
The proximate principles and phytochemicals present in the dried *Stevia rebaudiana* leaves were done in triplicates and their mean value was calculated. This helped to get concordant results by reducing the deviations.

Antimicrobial activity of *Stevia rebaudiana*

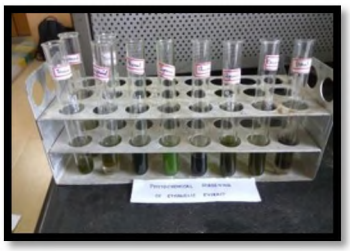
Stevia rebaudiana leaf infusion was subjected to antimicrobial activity by measuring the Diameter of Zone of Inhibition (IZD). Paper discs impregnated with specific antibiotics or the test substances were placed on the surface of the Muller Hinton Agar or Rose Bengal chloramphenicol inoculated with the target organisms. The plates were incubated and the zones of inhibition around each disc were measured. The procedure followed for the antimicrobial activity of *Stevia rebaudiana* leaves is shown in Appendix I and depicted in Plate 4.

Preparation of the extracts

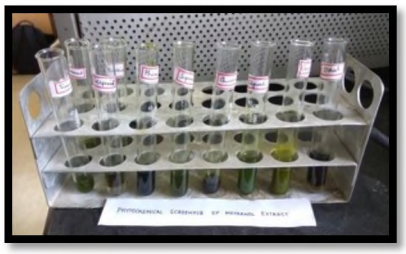
Aqueous, ethanol and methanol extracts of *Stevia rebaudiana* leaves



Aqueous extract



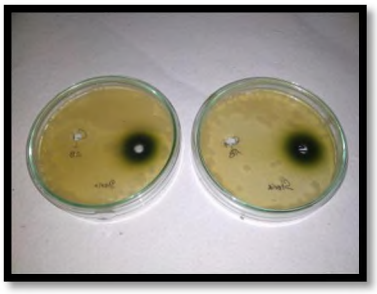
Ethanol extract



Methanol extract

Plate 3

Phytochemical analysis of *Stevia rebaudiana* leaves



Antibacterial activity

Antifungal activity

Plate 4

Antimicrobial activity of *Stevia rebaudiana* leaves

Analysis of toxic substances

Aflatoxins- Aflatoxin is determined by LC-MS, a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. Even with a very sophisticated MS instrument, HPLC is still useful to remove the interferences from the sample that would impact the ionization. Interface that will eliminate the solvent and generate gas phase ions, then transferred to the optics of the mass spectrometer (FSSAI, 2012).

Ochratoxin- Test portion is extracted by blending with acetonitrile–water. The extract is cleaned up by passing through an immunoaffinity column. Ochratoxin A (OTA) is eluted with methanol, further purified and identified by LC, and quantified by fluorescence (Entwisle *et al.*, 2000).

The procedure for the analysis of toxic substances is given in Appendix II.

B. Development and standardization of beverages and desserts substituted with *Stevia rebaudiana* leaves

Selection of recipes

Stevia rebaudiana leaves are rich in nutrients, containing substantial amount of protein, magnesium, zinc, chromium, selenium, calcium and phosphorus. Besides, it can also be used as a house hold sweetener in the preparation of most Indian beverages and sweets. *Stevia rebaudiana* leaves benefits the diabetic patients, either in dried or powdered form. In food products, it would not only aid in increasing the natural sweetness but would also help in rejuvenating the pancreatic gland (Choudhary *et al.*, 2014). According to Genus (2007), intake of dried *Stevia rebaudiana* leaves in amounts of 5 to 15 g per day is considered safe and ADI up to 250 mg dried leaves/kg body weight is suggested.

Stevia rebaudiana can be used as a safe and effective substitute for sugar in all recipes where sugar and low calorie sweeteners are normally used. Coffee and tea are the common beverages that are consumed by people all over the world in particular the people of Tamil Nadu to rejuvenate themselves.

According to a survey conducted by Hanspal (2010), the proportion of people consuming the beverage increased in 2005 to 62 per cent from 59

per cent in 2003. Fifty two per cent preferred instant coffee and 15 per cent used filter coffee. And most of the daily consumption of tea and coffee was at home. Similarly, in India, traditionally, every occasion is celebrated with the intake of desserts and it is also conventional to sweeten the mouth after every meal, festival, ceremony, social gatherings, offerings to God, etc., the consumption of desserts with a high concentration of sugar and fat in India is high (Gulati and Misra, 2014).

To facilitate the consumption of beverages like coffee and tea by the diabetics and those who are calorie conscious, substitution of dried *Stevia rebaudiana* leaves instead of sugar is the best way. Hence the beverages namely tea and coffee was selected for the study. Also, the commonly consumed desserts in India like rava kheer and paruppu payasam was selected for substitution.

Standardization of selected beverages and desserts substituted with *Stevia rebaudiana* leaves

The dried *Stevia rebaudiana* leaves were used as such in the preparation of beverages such as the tea and coffee. Various methods of substitution was tried to standardize the beverages. Initially weighed amounts of *Stevia rebaudiana* leaf powder was substituted in place of sugar in tea; directly added in the preparation of tea along with 2g tea powder, 50 ml of milk and 100 ml of water. This resulted in a strong bitter taste that had a greenish brown colour which was not acceptable for consumption.

Repeated trials with different methods were conducted with substitution of sugar using *Stevia rebaudiana* leaves as such, leaf in the form of powder, etc. These methods of using leaves or powder directly into the recipe resulted in either more sweetness or bitter taste or after taste etc. Finally, infusion method was tried and it had a good flavour and taste blended along with the product.

Finally, *Stevia rebaudiana* leaf infusion was prepared by steeping weighed amounts of *Stevia rebaudiana* leaf powder in hot water (95^{o}C) for 24 hours and filtered using a muslin cloth to remove the residues that gave the bitter after taste. The infusion was thus standardized and was used in the preparation of beverages and desserts.

Coffee was standardized by substituting *Stevia rebaudiana* leaves infusion. The standard (S) coffee was prepared using 100 ml of milk, 12.5 g of sugar and 2g of coffee powder. Three variations I, II and III were done by substituting 5ml, 10 ml and 15 ml of *Stevia rebaudiana* leaves infusion in coffee for sugar.

Tea was standardized by substituting *Stevia rebaudiana* leaves infusion into the normal tea preparation instead of sugar. The standard (S) tea was prepared using 125 ml of water, 50 ml of milk, 12.5 g of sugar and 2g of tea powder. Three variations I, II and III were done by substituting 5ml, 10 ml and 15 ml of *Stevia rebaudiana* leaves infusion in tea instead of sugar. Coffee and tea were prepared as per standard procedures.

For the development of desserts, initially dried leaf powder, as such was substituted in recipes at the cooking stage, which resulted in a strong bitter taste. An attempt was made by adding the *Stevia rebaudiana* leaves infusion at the cooking stage of the desserts instead of sugar which also gave bitterness. Hence the standardized infusion was added to the desserts after the preparation.

Rava kheer was prepared by using 20 g of rava, 100 ml of milk, 250 ml of water, and 25 g of sugar as the standard. Three variations I, II and III were done by substituting 7.5 ml, 15 ml and 22.5 ml of *Stevia rebaudiana* leaf infusion in rava kheer in place of sugar.

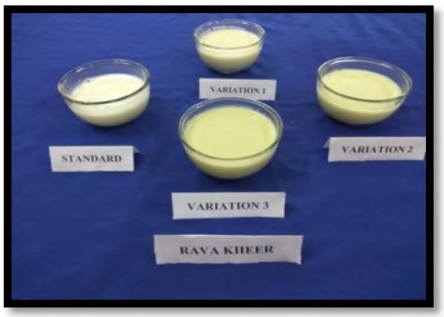
Moong dhal payasam was prepared by using 20 g of moong dal, 100 ml of milk, 250 ml of water and 30 g of jaggery as the standard. Three variations I, II and III were done by substituting 7.5 ml, 15 ml and 22.5 ml of *Stevia rebaudiana* leaf infusion for jaggery.

The development of beverages and desserts substituted with *Stevia rebaudiana* leaves is shown in Plate 5. The standardized recipe procedure of the desserts prepared are given in the Appendix III.

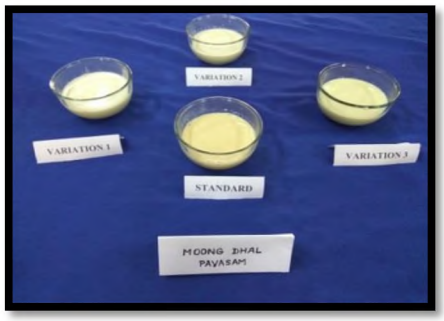
Coffee substituted with
Stevia rebaudiana leaves

Tea substituted with
Stevia rebaudiana leaves

Preparation of the *Stevia rebaudiana*
leaf substituted recipes



Rava kheer substituted with
Stevia rebaudiana leaves



Moong dhal payasam substituted
with *Stevia rebaudiana* leaves

Plate 5

Development of beverages and desserts substituted with *Stevia rebaudiana* leaves

C. Evaluation of organoleptic characteristics of *Stevia rebaudiana* leaves substituted beverages and desserts

Quality is a very important parameter for judging the edible nature of any food (Sharma, 2006). Sensory evaluation is a scientific discipline that uses the human senses, smell, sight, taste, touch and hearing which evokes, measures, analyses and interprets responses to products which influence the quality of the product (Sidel and Stone, 1993). Judging food quality is a matter of panelist's reaction to the sensory qualities of foods and is not a property of the food itself. Psychological and social factors play an important role in acceptance. Appearance, colour, flavour and mouth feel decide the acceptance of the food (Sethi *et al.*, 2001). Hence, sensory evaluation of the selected beverages and desserts substituted with *Stevia rebaudiana* leaves was carried out.

Sensory evaluation room is an essential parameter for carrying out the sensory analysis. The Foods Laboratory in the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore was selected as the venue for sensory evaluation.

Score card is a tool which helps in evaluation through direction and degree of judgement using suitable defined scores. Scoring is a form of rating of the prepared food items using a numerical scale (Manay and Shadaksharaswamy, 2007). A score card with 9 point hedonic scale, rating from like extremely to dislike extremely was prepared with the attributes like appearance, colour, flavour, texture, taste and overall acceptability of the recipes.

Panel members are a group of testers chosen to participate in a sensory test who are requested to assess food quality of samples presented for evaluation.

Thirty semi-trained panel members were selected based on their health, co-operation, willingness and knowledge of quality characteristics and sensory analysis of foods. The panel members involved in the study are the research scholars and post graduate students from the Departments of Food Science and Nutrition and Food Service Management and Dietetics who have knowledge about the sensory evaluation and its criteria to be assessed.

The recipes were prepared in the Foods Laboratory of the Department of Food Science and Nutrition of Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore. The two beverages tea and coffee and two desserts rava kheer and moong dhal payasam substituted with *Stevia rebaudiana* leaves was presented to the panel members for sensory evaluation at different points of time and were evaluated immediately after preparation, in order to prevent any changes in their quality, temperature changes and reheating. The sensory evaluation for the developed beverages and desserts were done in the morning between 10 and 11:30 am. The portion sizes for all the products were kept consistent and uniform.

Each panel member was allowed to take their own time to assess the product presented to them, during the sensory evaluation. The prepared products were neatly arranged and labelled as Standard, Variation I, Variation II and Variation III and was presented before the panel members. The panel members are provided with an evaluation form and a pen. A glass of water was also placed to enable the evaluator to rinse the mouth between tasting.

Acceptability and organoleptic scoring of the preparations was done on the basis of the scores given by the panel members. The scores obtained through sensory evaluation of the products by the panel members are recorded and the mean scores are calculated for each product. The overall acceptability and the mean scores for each product was analyzed and the product which obtained the highest total and mean score was considered to be the best acceptable.

The sensory evaluation of the beverages and desserts substituted with *Stevia rebaudiana* is exhibited in Plate 6 and the formulated score card is given in Appendix IV.

Coffee substituted with *Stevia rebaudiana* leaves

Tea substituted with *Stevia rebaudiana* leaves

Stevia rebaudiana leaf infusion

Rava kheer substituted with *Stevia rebaudiana* leaves

Moong dhal payasam substituted with *Stevia rebaudiana* leaves

Plate 6

Sensory evaluation of beverages and desserts substituted with *Stevia rebaudiana* leaves

D. Determination of glycemic index for the *Stevia rebaudiana* leaves substituted desserts

Glycemic index is a concept that is based on glycemic potency of foods (Dodd *et al.,* 2011) ranking the foods according to how quickly they raise the blood glucose levels. It is ratio of the incremental Area Under the Curve (iAUC) for blood glucose after consumption of a test food to the (iAUC) of a reference food containing the same amount of carbohydrate (Dodd *et al.,* 2011)

The glycemic index was calculated by ratio of the incremental area under curve for the test food to the incremental area under curve for the reference food and multiplied by 100 (Jenkins *et al*., 1981). Formula for calculating area under curve is given below:

$$\text{Glycemic index} = \frac{\text{iAUC of test food}}{\text{iAUC of reference food}} \times 100$$

(Tabassum *et al.,* 2013)

Glycemic response was assessed by finger prick method and it reflects the blood glucose level. Glucometer (Accu-chek Active) was used for the analysis. First, the finger was wiped using alcohol swabs, pricked with the lancet using a lancing device (Accu-chek Softclix). A drop of blood was applied on the glucose test strip. The glucose test strip after calibration, displays the blood glucose level in the display unit of the glucometer. The reading thus appeared was recorded.

Ten healthy individuals were selected for the glycemic index test for each dessert and the glycemic index was done on three consecutive days. The tests were conducted in the morning between 7 am to 10 am. On the first day, initially fasting blood glucose level was measured. The reference food i.e., 50g available carbohydrates in the form of white bread was given to the subjects along with water. The blood samples was drawn and checked for every 30, 60, 90 and 120 min for the postprandial glucose level.

On the second and third day, similarly fasting blood glucose level was measured after which measured portions of the standard and test food (*Stevia rebaudiana* leaves substituted desserts) containing 50 g of available

carbohydrate was fed to each subject respectively. The blood samples was drawn and checked for every 30, 60, 90 and 120 min for the postprandial glucose level. The reading obtained was used to construct a blood glucose response curve for the 2 hour period and thus the glycemic index was calculated. The glycemic index study of the developed desserts substituted with *Stevia rebaudiana* leaves is depicted in Plate 7.

The research design of the study is presented in Figure 1. The development and standardization of beverages and desserts substituted with *Stevia rebaudiana* leaves is presented in Figure 2.

E. Statistical analysis and interpretation of the data

The data was consolidated, tabulated and analyzed statistically to evaluate the sensory characteristics of the developed beverages and desserts substituted with *Stevia rebaudiana* leaves.

Ethical clearance

The study was presented in the Institutional Human Ethics Committee, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and the approval was obtained. The human ethical clearance approval number is AUW/ IHEC/ FSN -17-18/XPD/17 and the IHEC approved form is appended in the Appendix V.

Conduct of glycemic index test

Measuring the blood glucose levels using glucometer

Plate 7

Glycemic index study of the developed desserts substituted with *Stevia rebaudiana* leaves

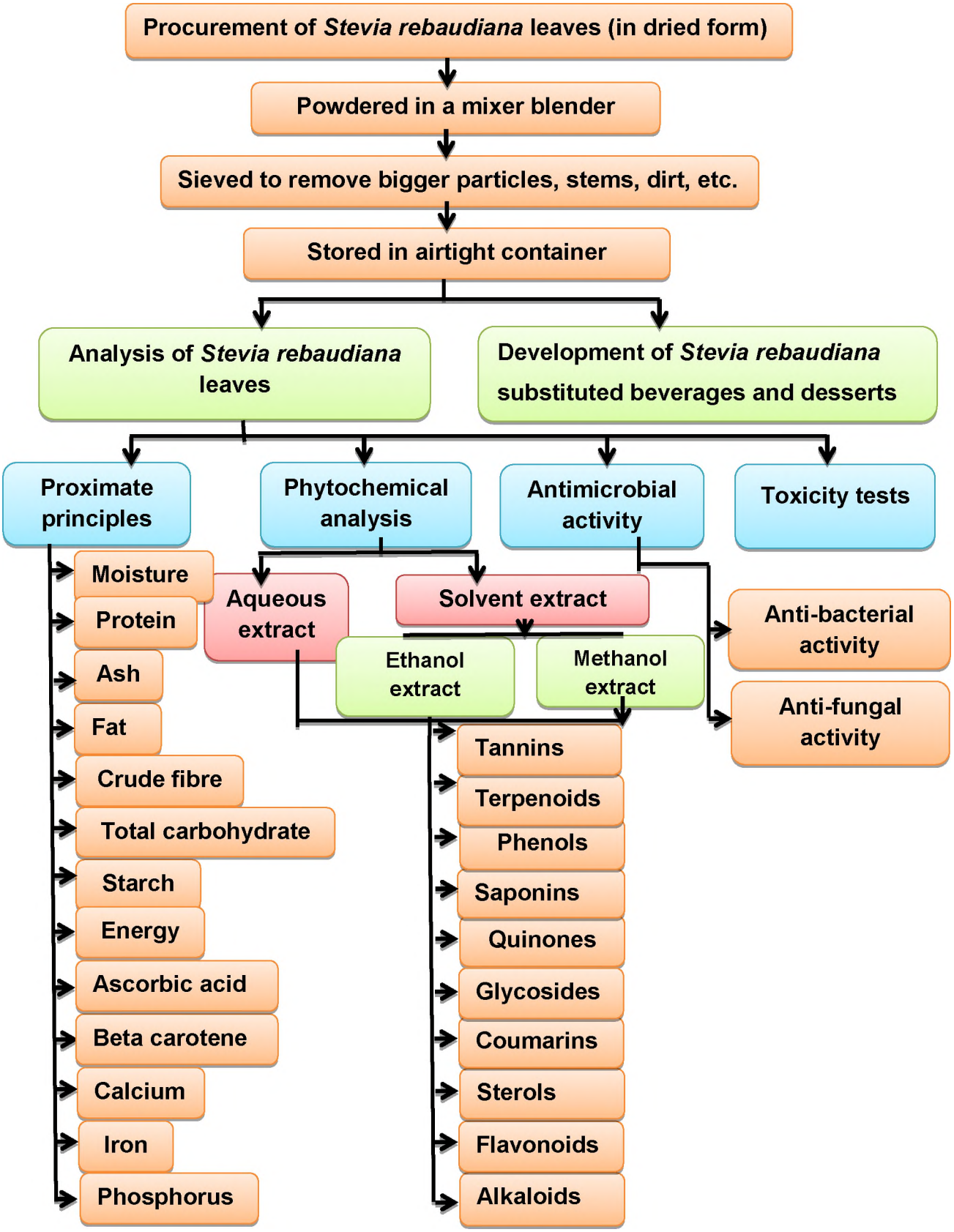


FIGURE 1

Research Design

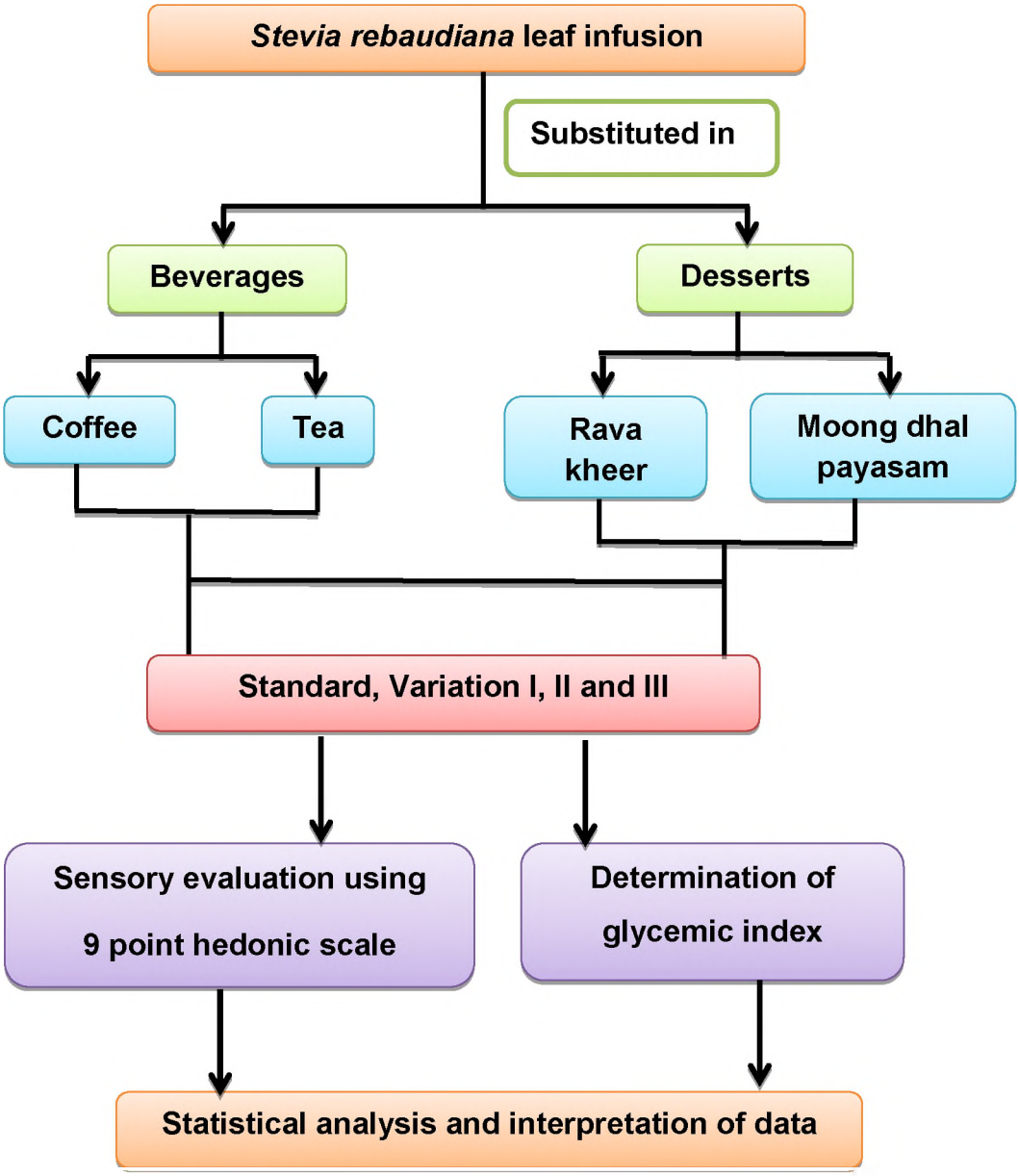


FIGURE 2

Development of desserts and beverages substituted with *Stevia rebaudiana* leaves

IV. RESULTS AND DISCUSSION

The results and discussion pertaining to the study "**Evaluation and development of beverages and desserts substituted with *Stevia rebaudiana* leaves**" is presented under the following headings:

A. Proximate principles of *Stevia rebaudiana* leaves
B. Phytochemical analysis of *Stevia rebaudiana* leaves
C. Antimicrobial activity and toxic substances in *Stevia rebaudiana* leaves
D. Sensory evaluation of beverages and desserts substituted with *Stevia rebaudiana leaves*
E. Glycemic index of desserts substituted with *Stevia rebaudiana* leaves

A. Proximate principles of *Stevia rebaudiana* leaves

The nutrient content of shade dried *Stevia rebaudiana* leaves is depicted in Table I.

TABLE I

QUANTITATIVE ANALYSIS OF PROXIMATE PRINCIPLES IN *STEVIA REBAUDIANA* LEAVES

Proximate principles	**Values** (per 100 g dry weight basis)
Moisture (%)	3.53 ± 0.80
Protein (g)	16.66 ± 3.60
Ash (g)	10
Fat (g)	4.66 ± 1.15
Crude fibre (g)	11.58 ± 0.34
Carbohydrate (g)	32.50 ± 2.50
Starch (g)	1.66 ± 0.15
Energy (kcal)	238.66 ± 10.78
Ascorbic acid (mg)	30.95 ± 3.34
Beta carotene (μg)	213.33 ± 23.09
Calcium (mg)	726.66 ± 6.11
Iron (mg)	11.93 ± 0.11
Phosphorus (mg)	386.66 ± 11.54

Each value represents the mean ± SD of three determinations on dry weight basis

The moisture content of *Stevia rebaudiana* leaves was 3.53 % per 100 g of leaves. The protein content was 16.66 g, ash content with 10 g, fat content with 4.66 g and crude fibre with 11.58 g per 100 g of the leaves. The carbohydrate content of *Stevia rebaudiana* leaves was 32.50 g and starch present in 1.66 g per 100g of the leaves. The energy value of *Stevia rebaudiana* leaves per 100g was 238.66 kcal. Vitamins and minerals which were analyzed include ascorbic acid, beta carotene, calcium, iron and phosphorus. The ascorbic acid present in 100 g of *Stevia rebaudiana* leaves was 30.95 mg.

Carotenoid has antioxidant activity and prevents cancer, cardiovascular disorders and other diseases. Beta carotene has the high antioxidant activity among the carotenes (Stahl and Sies, 2005). Beta carotene was in the level of 213.33 μg per 100 g of the leaves. The calcium content was found to be 726.66 mg. Iron is a major component of the cytochromes functioning in cellular respiration and RBCs require iron in haemoglobin, for the well-functioning (Soetan *et al.,* 2010). The iron content of *Stevia rebaudiana* leaves was11.93 mg.

Phosphorus is a significant constituent of adenosine triphosphate (ATP) and nucleic acid and crucial for acid-base balance, bone and tooth formation (Soetan *et al.,* 2010). The phosphorus content of *Stevia rebaudiana* leaves were 386.66 mg per 100 g.

The moisture values of dried *Stevia rebaudiana* leaves found out in other studies were 4.65 g (Goyal *et al*., 2010), 7.7 g (Kaushik *et al*., 2010) and 7 g (Mishra *et al*., 2010) respectively.

Protein content was 20.4 g (Tadhani and Subash, 2006), 12 g (Kaushik *et al*., 2010), 11.2 g (Goyal *et al*., 2010; Serio, 2010) and 10 g (Mishra *et al*., 2010). The present finding shows protein content in between these values. The ash content of the studies lie in the range of 6.3g, 8.4 g ,11 g and 13.1 g found by Goyal *et al*., 2010; Kaushik *et al*., 2010; Mishra *et al*., 2010 and Tadhani and Subash, 2006 respectively.

Fat content found out by Goyal *et al*., 2010 (1.9 g), Kaushik *et al*., 2010 (2.7 g), Mishra *et al*., 2010 (3 g), Tadhani and Subash, 2006 (4.34 g) and Serio, 2010 (5.6 g) per 100 g dry weight basis. The crude fibre were found as 15 g, 15.2 g and

18 g by Serio, 2010, Goyal *et al.*, 2010 and Mishra *et al.*, 2010 per 100 g on dry weight basis. Carbohydrate content of *Stevia rebaudiana* leaves per 100 g dry weight basis were 35.2 g, 52 g and 53 g found by Tadhani and Subash, (2006), Mishra *et al.*, (2010) and Serio, 2010 respectively.

Ascorbic acid was found out to be 29 mg (Gupta *et al.*, 2013) and 14.97 mg (Kim *et al.,* 2011) per 100g and beta carotene with 200 μg (Gupta *et al.*, 2013) per 100 g of dry weight. Calcium was found out to be 728 mg per 100g by Goyal *et al.*, (2010). Iron was in the range of 12 mg as reported by Tadhani and Subash (2006). Phosphorus content was found to be 380 mg per 100 g.

B. Phytochemical analysis of *Stevia rebaudiana* leaves

The qualitative analysis of phytochemicals in *Stevia rebaudiana* leaves is depicted in the Table II.

TABLE II

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN *STEVIA REBAUDIANA* LEAVES

Phytochemical	Solvent		
	Aqueous Extract	Ethanol Extract	Methanol Extract
Tannins	++	+	++
Terpenoids	+++	+++	+++
Phenols	+	+	++
Saponins	+++	-	-
Quinones	++	-	+
Glycosides	+++	+++	+++
Coumarins	+++	+	++
Sterols	+++	+++	+++
Flavanoids	++	+	++
Alkaloids	++	+	+

+++ Strongly present, ++ Present, + Weakly present, - Absent

Phytochemicals play a crucial role in preventing and managing of modern diseases such as cancers, diabetes, Alzheimer's diseases and cardiovascular diseases (Andrae-Marobela *et al.*, 2013). Qualitative analysis of phytochemicals was done in aqueous extract, solvent extracts viz. ethanol and methanol of *Stevia rebaudiana* leaves. The various extracts were screened for phytochemicals like tannins, terpenoids, phenols, saponins, quinones, glycosides, coumarins, sterols, flavonoids and alkaloids.

The aqueous extract of *Stevia rebaudiana* leaves showed a strong presence of phytochemicals like terpenoids, saponins, glycosides, coumarins and sterols. Tannins, quinones, flavonoid and alkaloids were present in aqueous extract; while phenols were weakly present in aqueous extract.

The ethanol extract of *Stevia rebaudiana* leaves indicated the strong presence of terpenoids, glycosides and sterols. The phytochemicals such as tannins, phenols, coumarin, flavonoids and alkaloids were weakly present in the ethanol extract of *Stevia rebaudiana* leaves. Saponins and quinones were absent in the ethanol extract of *Stevia rebaudiana* leaves.

The methanol extract of *Stevia rebaudiana* leaves indicated a strong presence of terpenoids, glycosides and sterols. The methanol extract showed the presence of phytochemicals such as tannins, phenols, coumarins and flavonoids. The phytochemicals such as glycosides and flavonoids were weakly present in methanol extract of *Stevia rebaudiana* leaves whereas saponins was completely absent in methanol extract of *Stevia rebaudiana* leaves.

This finding correlates with the reports of Kujur *et al.,* (2010) in which the aqueous extract of *Stevia rebaudiana* leaves showed a strong presence of sterols, presence of alkaloids, flavonoids and weak presence of phenols and traces of saponins and tannins. The results of the present study on qualitative phytochemical analysis of *Stevia rebaudiana* leaves in ethanol extract correlate well with findings of Sheeja and Lawrence (2015) wherein the aqueous extract has strong presence of alkaloids, glycosides and tannins and presence of flavonoids and terpenoids; and the phytochemicals such as saponins were present in weak amounts and absent in coumarins and quinones. Kujur *et al.,* (2010) showed that the methanol extracts of *Stevia rebaudiana* leaves had a strong presence of phenols; weak

presence of flavanoids, saponins, sterols and tannins strongly present and absence of alkaloids which is controversy to the present study.

From the various extracts done in *Stevia rebaudiana* leaves which were screened for phytochemicals, the terpenoids, glycosides and sterols were strongly present in all the three extracts viz. aqueous and solvents like ethanol and methanol extract. Out of the three extracts screened for the phytochemicals, the aqueous extract showed the maximum presence of all the phytochemicals thus exhibiting the potentials of phytochemicals in *Stevia rebaudiana* indicating as a most acceptable herb rich in phytochemicals.

C. Antimicrobial activity and toxic substances in *Stevia rebaudiana* leaves

The antimicrobial activity of *Stevia rebaudiana* leaves of aqueous extract is depicted in Table III.

TABLE III

ANTIMICROBIAL ACTIVITY OF *STEVIA REBAUDIANA* LEAVES

Antimicrobial activity	Microorganism	Zone of inhibition (mm)	
		Aqueous	Control
Bacteria	*Escherichia coli*	9.0	8.5
	Staphylococcus aureus	11.0	9.5
Fungi	*Aspergillus flavus*	11.5	6.0
	Aspergillus niger	13.0	7.0

The aqueous extract of *Stevia rebaudiana* leaves were subjected to antimicrobial activity namely antibacterial and antifungal activity. The antibacterial activity was done with gram negative bacteria namely *Escherichia coli* and a gram positive bacteria namely *Staphylococcus aureus*.

The antibacterial activity of aqueous extract of *Stevia rebaudiana* leaves showed a zone of inhibition of 9.0 mm which was greater than that of control with 8.5 mm against *Escherichia coli*. The zone of inhibition was 11.0 mm against *Staphylococcus aureus* higher than that of the control with 9.5 mm in aqueous

extract of *Stevia rebaudiana* leaves. The antibacterial activity of the present study was greater than that of the findings by Tadhani and Subash, (2006) with a zone of inhibition of 9.33 mm in aqueous extract of *Stevia rebaudiana* leaves.

The antifungal activity was studied in aqueous extracts of *Stevia rebaudiana* leaves against fungi namely *Aspergillus flavus* and *Aspergillus niger*. The control *Aspergillus flavus* showed zone of inhibition of 6.0 mm whereas the aqueous extract of *Stevia rebaudiana* leaves showed a greater zone of inhibition of 11.5 mm than the control. The zone of inhibition of *Stevia rebaudiana* leaf extract against *Aspergillus flavus* was observed to be 13.0 mm which was significantly higher than the control with 7.0 mm. The antifungal activity of *Stevia rebaudiana* leaves against fungi was observed to be higher the findings of Ghosh *et al.,* (2008) with zone of inhibition of 11.0 mm against *Aspergillus niger*.

The results of this study indicate that the *Stevia rebaudiana* leaf extract have inhibitory activities against microorganisms, as they have significantly higher activity than the control.

Toxic substances in *Stevia rebaudiana* leaves

Table IV shows the toxic substances present in *Stevia rebaudiana* leaves.

TABLE IV

TOXIC SUBSTANCES IN *STEVIA REBAUDIANA* LEAVES

Toxin	Detected level ($100\ g^{-1}$ on dry weight basis)
Aflatoxin	9.0 ppb
Ochratoxin	BDL

ppb- parts per billion, BDL- Below Detectable Level (0.001%)

The post-harvest storage of certain herbs and farm produce have potential to help growth of microorganisms on them that leads to decrease the shelf-life, augmenting food safety issues due to allergens and pathogens (Ranjan *et al.,* 2014).

Mycotoxins are the fungal metabolites that evoke pathological changes in humans. They affect human populations and result in diseases. The major

mycotoxins are aflatoxin and ochratoxin that are harmful to health if they exceed the action levels (Mazumder and Sasmal, 2001). The *Stevia rebaudiana* leaves were subjected to detection of toxic substances such as aflatoxin and ochratoxin.

According to USDA, (2015) the action level of aflatoxin is 20 ppb in foods. The *Stevia rebaudiana* leaves contained aflatoxin in the quantities of 9.0 ppb which is lesser than the action level. Ochratoxin was found to be below the detection limit. The results clearly shows that *Stevia rebaudiana* leaves are safe for consumption since the content of the toxins lie in the safe upper limit.

In a study by Ranjan *et al.,* (2014) the aflatoxin content in the *Stevia rebaudiana* leaves exceeded the safe limit than the present study which was well within the control limit and may be due to the location of cultivation, storage conditions more susceptible to the microbial growth.

D. Sensory evaluation of beverages and desserts substituted with *Stevia rebaudiana leaves*

A food may be nutritious and economic but the key to a successful product is that it must smell, look, feel and taste good (Vieira, 1996). The sensory aspects of food are measured by intensities of taste, flavor profile evaluation, food texture, and consistency and appearance assessment as devised by experimental psychologists. Important attitudinal and socioeconomic factors are likely to be involved in hedonic rating in addition to sensory variables and a sensory evaluation must work along market research to satisfy consumer acceptance and community health needs (Drewnowski and Moskow, 1985).

Two popular beverages consumed regularly atleast twice in a day in normal routine and two desserts that were commonly consumed were selected for the substitution with *Stevia rebaudiana* leaves instead of sugar. The selected beverages coffee and tea were substituted with *Stevia rebaudiana* leaves infusion in quantities of 5 ml, 10 ml and 15 ml for variation I, II and III respectively. The selected desserts rava kheer and moong dhal payasam were subjected to variation I, II and III substituting *Stevia rebaudiana* leaf infusion of 7.5 ml, 15 ml and 22.5 ml respectively.

Sensory evaluation of coffee substituted with *Stevia rebaudiana leaves*

Table V and Figure 3 posturizes the sensory evaluation of coffee substituted with *Stevia rebaudiana* leaves.

TABLE V

SENSORY EVALUATION OF COFFEE SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

N= 30

Criteria	Standard	Variation I	Variation II	Variation III
Appearance	8.86 ± 0.34	8.63 ± 0.55	8.20 ± 0.80	7.70 ± 1.14
Colour	8.83 ± 0.53	8.36 ± 0.71	8.00 ± 0.90	7.76 ± 1.13
Flavour	8.83 ± 0.53	8.16 ± 0.69	7.73 ± 0.98	7.00 ± 1.14
Consistency	8.86 ± 0.34	8.26 ± 0.73	7.80 ± 0.88	7.06 ± 1.01
Taste	8.83 ± 0.37	7.80 ± 0.71	7.16 ± 0.87	6.50 ± 1.00
Overall acceptability	8.83 ± 0.37	7.93 ± 0.69	7.50 ± 0.68	6.66 ± 1.02
F value Standard *vs* variation		3.930**	3.500^{NS}	1.246^{NS}

** - Significant at 5% level, ^{NS} - Not significant

Sensory evaluation of coffee substituted with *Stevia rebaudiana* leaves revealed that the appearance for standard was 8.86, variation I, 8.63, variation II, 8.20 and variation III was 7.70. The mean values obtained for colour was 8.83 for standard, 8.36 for variation I, 8.00 for variation II and 7.76 for variation III. The mean scores obtained for the flavour in coffee substituted with *Stevia rebaudiana* leaves ranged from 7.00 to 8.83, the maximum score obtained for standard with a mean value of 8.83 followed by variation I with 8.16, variation II with 7.73 and variation III with 7.00. With regard to consistency, standard showed the best result with a mean value of 8.86 followed by variation I with 8.26, variation II with 7.80 and variation III with 7.06. The taste of the standard scored maximum mean value of 8.83 followed by 7.80, 7.16 and 6.50 for variation I, variation II and variation III respectively.

The overall acceptability of coffee substituted with *Stevia rebaudiana* leaves revealed that variation I had a maximum mean score of 7.93 and was comparable with the standard of 8.83. This was followed by variation II and II with mean scores 7.50 and 6.66 respectively. It was observed that the overall acceptability of coffee with *Stevia rebaudiana* leaf infusion was statistically significant at 5 % level when standard and variation I was compared. No significant change was observed for overall acceptability for variation I, II and III.

Sensory evaluation of tea substituted with *Stevia rebaudiana leaves*

Table VI and Figure 4 posturizes the sensory evaluation of tea substituted with *Stevia rebaudiana* leaves.

TABLE VI

SENSORY EVALUATION OF TEA SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

N= 30

Criteria	Standard	Variation I	Variation II	Variation III
Appearance	8.96 ± 0.18	8.80 ± 0.48	8.56 ± 0.56	8.13 ± 0.97
Colour	8.96 ± 0.18	8.73 ± 0.52	8.53 ± 0.50	7.96 ± 0.92
Flavour	8.90 ± 0.40	8.06 ± 0.78	8.03 ± 0.85	7.10 ± 1.12
Consistency	8.93 ± 0.36	8.36 ± 0.66	8.36 ± 0.66	7.63 ± 1.06
Taste	8.93 ± 0.25	7.96 ± 0.66	7.90 ± 0.84	6.80 ± 0.96
Overall acceptability	8.96 ± 0.18	8.10 ± 0.71	8.00 ± 0.78	7.00 ± 0.94
F value Standard *vs* Variation		1.303^{NS}	0.854^{NS}	0.000^{NS}

** - Significant at 5% level, ^{NS} - Not Significant

From the above table, it was found that the appearance of the tea substituted with *Stevia rebaudiana* leaves for standard, variations I, II and III, standard scored the best result with mean value of 8.96, variation III scoring a decreased score of 8.13. In terms of colour, standard and three variations got

mean scores from 7.96 to 8.96. Variation I and II had a mean value of 8.73 and 8.53 respectively. The maximum mean score for colour was for standard with 8.96. The mean scores obtained for flavor ranged from 7.10 to 8.90, the minimum score 7.10 for variation III, 8.03 for variation II and 8.06 for variation I and 8.90 for standard. Regarding consistency, mean scores of standard showed a value of 8.93, variation I and variation II with 8.36 each and variation III with 7.63. Taste contributed mean scores from 6.80 to 8.93.The maximum mean value obtained for standard was 8.93 followed by variation I with 7.96, variation II with 7.90 and variation III with 6.80.

The mean overall acceptability score was 8.96 for standard; 8.10 for variation I; 8.00 for variation II and 7.00 for variation III respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III. It was observed that the overall acceptability was not significant when standard and variations were compared.

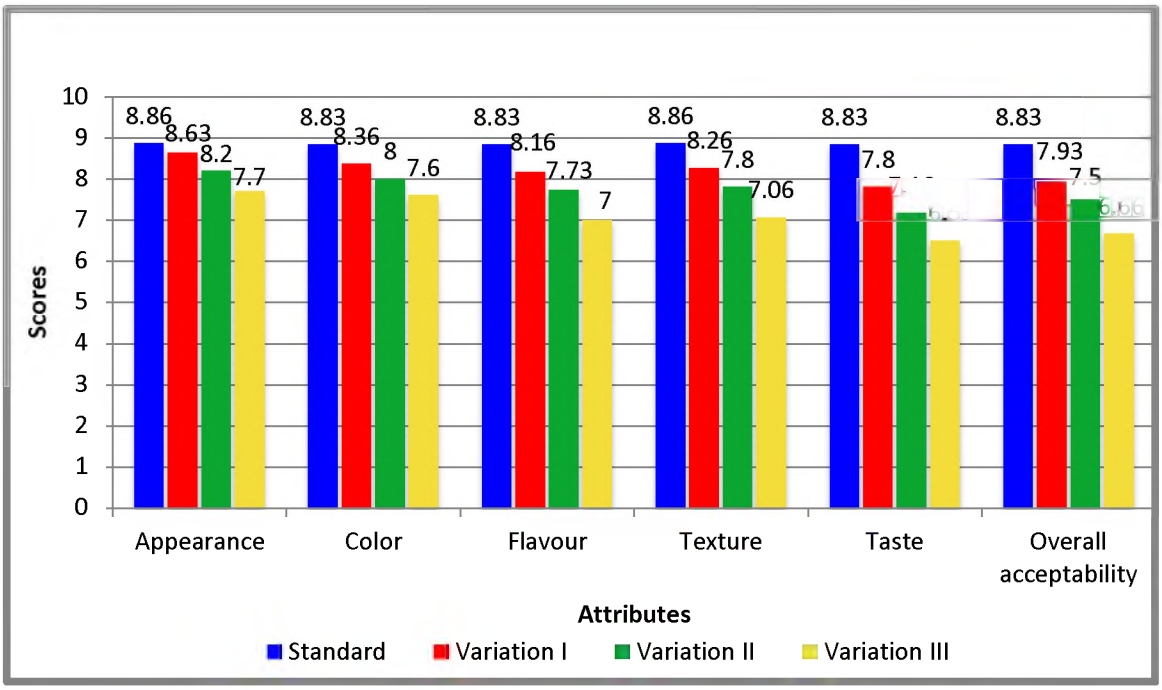


FIGURE 3

Sensory evaluation of coffee substituted with *Stevia rebaudiana leaves*

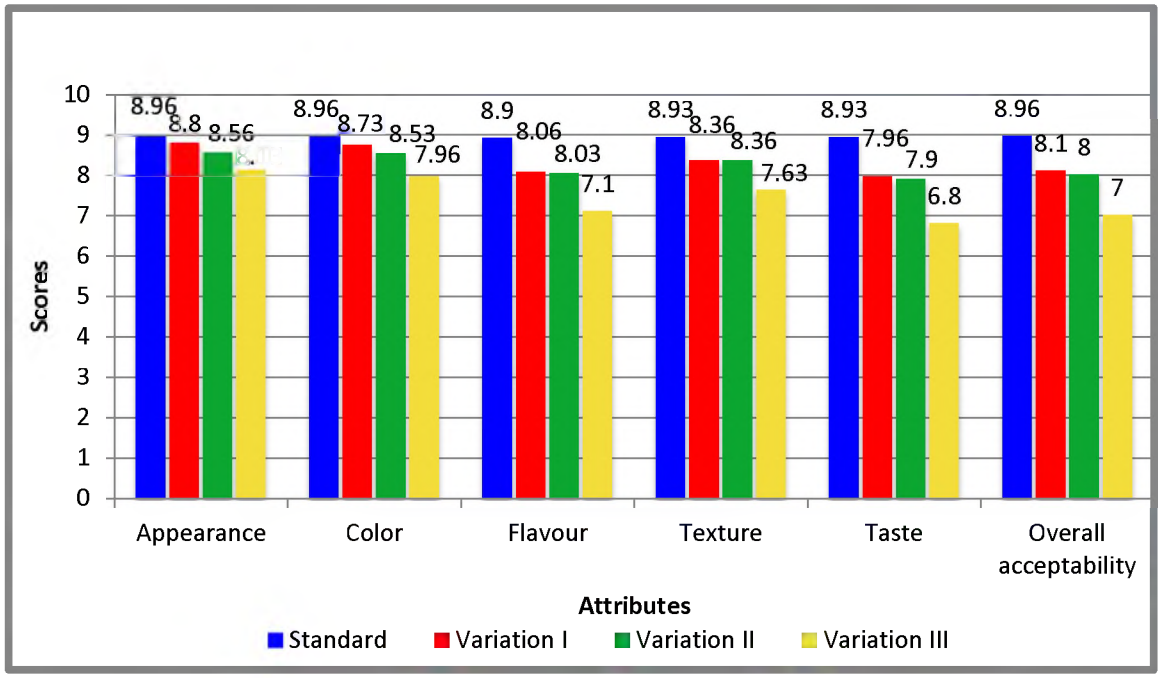


FIGURE 4

Sensory evaluation of tea substituted with *Stevia rebaudiana leaves*

Sensory evaluation of rava kheer substituted with *Stevia rebaudiana leaves*

Table VII and Figure 5 posturizes the sensory evaluation of rava kheer substituted with *Stevia rebaudiana* leaves.

TABLE VII

SENSORY EVALUATION OF RAVA KHEER SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

N= 30

Criteria	Standard	Variation I	Variation II	Variation III
Appearance	8.36 ± 0.61	8.10 ± 0.92	8.03 ± 0.80	7.46 ± 1.22
Colour	8.56 ± 0.56	8.13 ± 0.77	8.03 ± 0.85	7.26 ± 1.31
Flavour	8.46 ± 0.57	7.33 ± 1.12	7.16 ± 1.05	5.73 ± 1.63
Texture	8.26 ± 0.78	7.76 ± 0.93	7.83 ± 0.98	7.16 ± 1.14
Taste	8.56 ± 0.50	7.16 ± 1.14	6.70 ± 1.80	5.10 ± 1.97
Overall acceptability	8.56 ± 0.56	7.20 ± 1.21	7.10 ± 1.21	5.53 ± 1.63
F value Standard *vs* Variation		2.510 ^{NS}	2.302 ^{NS}	0.557 ^{NS}

** - Significant at 5% level, ^{NS} - Not Significant

From the above table, it is clear that the appearance of standard was maximum with mean value of 8.36 followed by variation I with 8.10, variation II with 8.03 and variation III with 7.46 for rava kheer substituted with *Stevia rebaudiana* leaves. Colour contributed mean scores from 7.26 to 8.56. The maximum mean score for colour obtained for standard with 8.56 followed by 8.13, 8.03 and 7.26 for variation I, II and III respectively. In terms of flavour and texture, standard got mean value of 8.46 and 8.26 respectively, variation I got 7.33 and 7.76 respectively, variation II got 7.16 and 7.83 respectively and variation III got 5.73 and 7.16 respectively. The taste of the standard, variation I, variation II and variation III secured a mean value of 8.56, 7.16, 6.70 and 5.10 correspondingly.

Overall acceptability of standard was 8.56, variation I was 7.20, variation II was 7.10 and variation III was 5.53 respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III.

The F values were 2.510, 2.302 and 0.557 for variation I, II and III respectively. The results implied that the variations were not significant statistically.

Sensory evaluation of moong dhal payasam substituted with *Stevia rebaudiana leaves*

Table VIII and Figure 6 posturizes the sensory evaluation of moong dhal payasam substituted with *Stevia rebaudiana* leaves.

TABLE VIII

SENSORY EVALUATION OF MOONG DHAL PAYASAM SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

N= 30

Criteria	Standard	Variation I	Variation II	Variation III
Appearance	8.83 ± 0.53	8.66 ± 0.54	8.63 ± 0.49	8.56 ± 0.81
Colour	8.90 ± 0.30	8.63 ± 0.55	8.53 ± 0.73	8.56 ± 0.72
Flavour	8.73 ± 0.58	7.80 ± 0.76	7.26 ± 1.08	5.80 ± 1.49
Texture	8.66 ± 0.66	8.43 ± 0.67	8.46 ± 0.62	8.23 ± 0.85
Taste	8.86 ± 0.34	7.76 ± 0.67	7.03 ± 1.12	5.46 ± 1.25
Overall acceptability	8.93 ± 0.25	7.90 ± 0.60	7.40 ± 1.19	5.56 ± 1.47
F value Standard *vs* Variation		0.079 ^{NS}	2.287 ^{NS}	0.098 ^{NS}

** - Significant at 5% level, ^{NS} - Not Significant

Table VIII showed the results of sensory evaluation of moong dhal payasam substituted with *Stevia rebaudiana* leaves. In terms of appearance, standard scored best with a mean score of 8.83 followed by variation I with 8.66, variation II with 8.63 and variation III with 8.56. In terms of colour, variation II had minimum score of 8.53 followed by variation III with 8.56, variation I with 8.63 and standard with 8.90. The mean scores obtained for the flavour in moong dhal payasam substituted with *Stevia rebaudiana* leaves ranged from 5.80 to 8.73, the maximum obtained by standard with a mean of 8.73 followed by 7.80, 7.26 and 5.80 for variation I, II and III respectively. Regarding texture, mean scores of standard

showed a maximum value of 8.66 followed by variation II with 8.46, variation I with 8.43 and variation III with 8.23 respectively. Taste is an important attribute. Taste contributes scores from 5.46 to 8.86 and maximum being in standard with 8.86 followed by variation I with 7.76, variation II with 7.03 and variation III with 5.46.

In terms of overall acceptability, standard obtained 8.93 followed by variation I with 7.90 mean score; variation II with 7.40 and variation III with 5.56 respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III. The F values were 0.079, 2.287 and 0.098 for variation I, II and III respectively. The results revealed that the variations are not significant statistically.

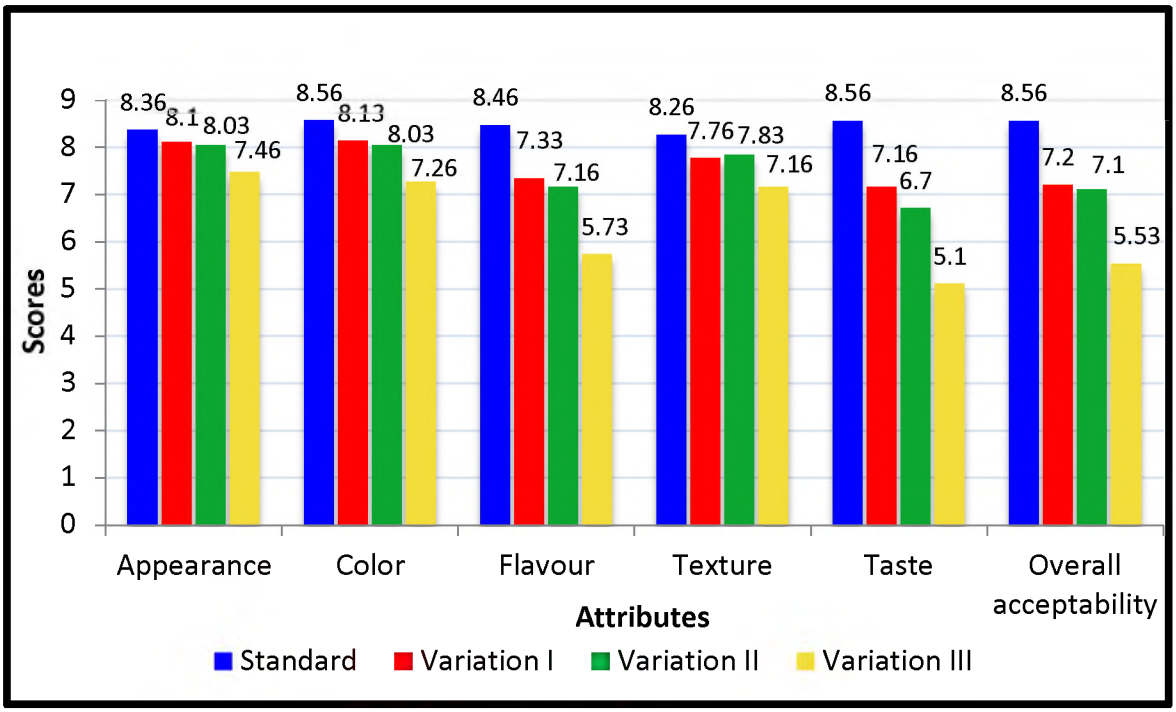


FIGURE 5

Sensory evaluation of rava kheer substituted with *Stevia rebaudiana leaves*

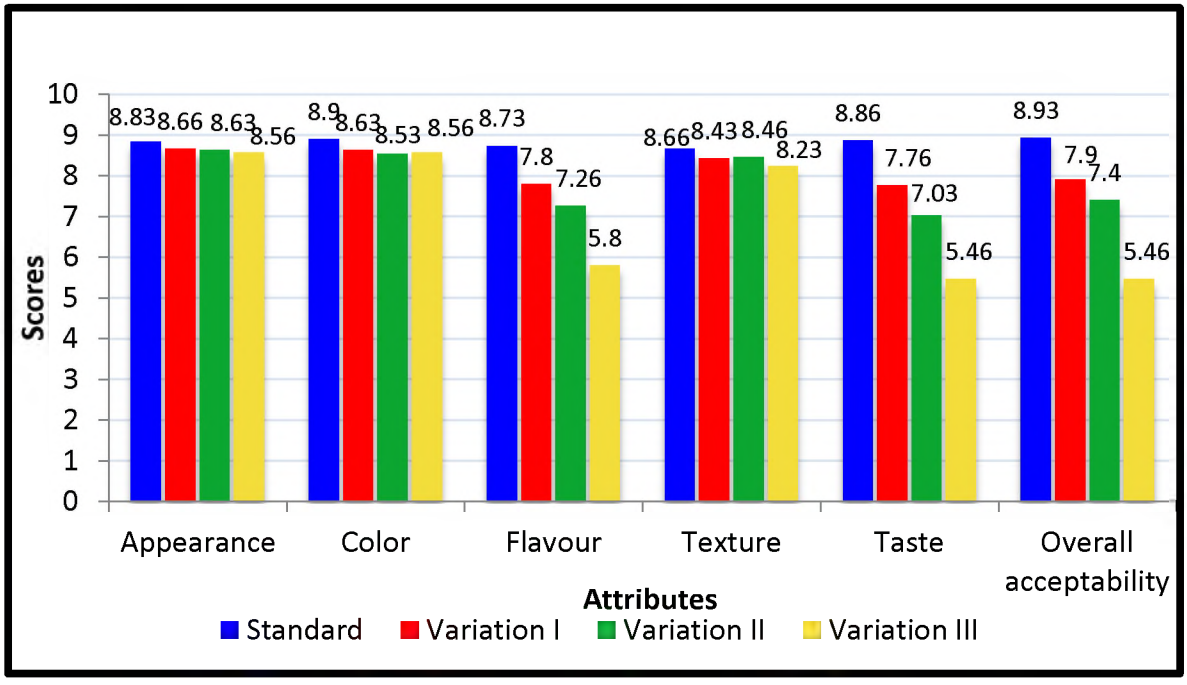


FIGURE 6

Sensory evaluation of moong dhal payasam substituted with *Stevia rebaudiana* leaves

Overall acceptability of beverages substituted with *Stevia rebaudiana* leaves

Table IX reveals the overall acceptability of beverages substituted with *Stevia rebaudiana* leaves.

TABLE IX

OVERALL ACCEPTABILITY OF BEVERAGES SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

Beverages	Standard	Variation I	Variation II	Variation III
Coffee	8.83 ± 0.37	7.93 ± 0.69	7.50 ± 0.68	6.66 ± 1.02
Tea	8.96 ± 0.18	8.10 ± 0.71	8.00 ± 0.78	7.00 ± 0.94

The overall acceptability of beverages substituted with *Stevia rebaudiana* leaves showed that, for coffee standard scored a mean maximum value with 8.83 followed by variation I with 7.93, variation II with 7.50 and variation III with 6.66. For tea, the maximum overall acceptability secured for standard with mean value of 8.96, variation I with 8.10, variation II with 8.00 and variation III with 7.00.

The overall results indicate that variation I with 5 ml *Stevia rebaudiana* leaf infusion instead of sugar had an overall acceptability when compared with variation II and variation III. Hence this level of substitution can be included in the beverages which may enhance the nutritional value of the beverage consumed.

Overall acceptability of desserts substituted with *Stevia rebaudiana* leaves

Table X reveals the overall acceptability of desserts substituted with *Stevia rebaudiana* leaves.

TABLE X

OVERALL ACCEPTABILITY OF DESSERTS SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

Desserts	Standard	Variation I	Variation II	Variation III
Rava kheer	8.56 ± 0.56	7.20 ± 1.21	7.10 ± 1.21	5.53 ± 1.63
Moong dhal payasam	8.93 ± 0.25	7.90 ± 0.60	7.40 ± 1.19	5.56 ± 1.47

The overall acceptability of desserts substituted with *Stevia rebaudiana* leaves showed that, for rava kheer, a score of 8.56 for standard with mean maximum score followed by variation I with 7.20, variation II with 7.10 and variation III with 5.53. The overall acceptability of moong dhal payasam secured 8.93 for standard, 7.90 for variation I, 7.40 for variation II and 5.56 for variation III.

The overall results indicate that variation I with 7.5 ml *Stevia rebaudiana* leaf infusion for sugar was best acceptable when compared with variation II and variation III. Hence this level of substitution can be included in the desserts. This may enhance the nutritional value of the desserts with a guilt free consumption of desserts for diabetics and calorie conscious consumers.

E. Glycemic index of desserts substituted with *Stevia rebaudiana* leaves

Glycemic response of rava kheer substituted with *Stevia rebaudiana* leaves

Table XI and Figure 7 depicts the glycemic response of rava kheer substituted with *Stevia rebaudiana* leaves.

TABLE XI

GLYCEMIC RESPONSE OF RAVA KHEER SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

Food product	Blood glucose level (mg dl^{-1})				
	Fasting	30 min	60 min	90 min	120 min
White bread	92.1 ± 5.5	135.7 ± 26.6	136.1 ± 35.6	126.0 ± 15.9	108.4 ± 11.5
Rava kheer Standard	89.8 ± 3.6	119.7 ± 32.4	107.5 ± 9.9	104.0 ± 9.6	101.8 ± 10.0
Rava kheer with *Stevia*	90.0 ± 3.6	115.3 ± 6.8	103.4 ± 7.3	102.3 ± 11.0	99.0 ± 4.7

Rava kheer substituted with *Stevia rebaudiana* leaves in the form of infusion (7.5 ml) containing 50 g available carbohydrate was given to healthy individuals and the glycemic response and the mean values are presented in Table XI. The

mean glycemic response of the reference food was found to be 92.1, 135.7, 136.1, 126.0 and 108.4 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The mean glycemic response of rava kheer prepared with sugar (standard) was 89.8, 119.7, 107.5, 104.0 and 101.8 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively and the mean glycemic response of rava kheer substituted with *Stevia rebaudiana* leaves in the form of infusion was 90.0, 115.3, 103.4, 102.3 and 99.0 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The peak value was 136.1 mg/dl for reference food observed at 60 min whereas the peak value was 119.7 and 115.3 mg/dl for standard rava kheer and *Stevia rebaudiana* substituted rava kheer at 30 min. Thereafter the levels showed a decreasing trend at 120 min. White bread recorded the highest response (108.4 mg/dl) followed by standard rava kheer (101.8 mg/dl) and the lowest response (99.0 mg/dl) by *Stevia rebaudiana* substituted rava kheer.

Glycemic response of moong dhal payasam substituted with *Stevia rebaudiana* leaves

Table XII and Figure 8 depicts the glycemic response of moong dhal payasam substituted with *Stevia rebaudiana* leaves.

TABLE XII

GLYCEMIC RESPONSE OF MOONG DHAL PAYASAM SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

Food product	Blood glucose level (mg/dl)				
	Fasting	30 min	60 min	90 min	120 min
White bread	90.4 ± 4.9	125.1 ± 36.4	113.1 ± 26.4	112.3 ± 22.4	103.7 ± 16.2
Moong dhal payasam Standard	92.3 ± 11.2	113.5 ± 29.9	98.9 ± 9.4	97.9 ± 7.4	93.2 ± 14.2
Moong dhal payasam with *Stevia*	86.1 ± 8.4	96.1 ± 13.6	93.9 ± 7.5	93.5 ± 20.6	92.4 ± 14.4

Moong dhal payasam substituted with *Stevia rebaudiana* leaves in form of infusion (7.5 ml) containing 50 g available carbohydrate was given to healthy

individuals whose glycemic response and the mean values are depicted in Table X. The mean glycemic response of the reference food was found to be 90.4, 125.1, 113.1, 112.3 and 103.7 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The mean glycemic response of moong dhal payasam prepared with sugar (standard) was 92.3, 113.5, 98.9, 97.9 and 93.2 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively and the mean glycemic response of moong dhal payasam substituted with *Stevia rebaudiana* leaves in the form of infusion was 86.1, 96.1, 93.9, 93.5 and 92.4 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The peak value was 125.1 mg/dl for reference food observed at 30 min whereas the peak value was 113.5 and 96.1 mg/dl for standard moong dhal payasam and *Stevia rebaudiana* substituted moong dhal payasam at 30 min respectively. Thereafter the levels showed a decreasing trend at 120 min for reference food and standard moong dhal payasam. White bread recorded the highest response (103.7 mg/dl) followed by standard moong dhal payasam (93.2 mg/dl) and the lowest response (92.4 mg/dl) by *Stevia rebaudiana* substituted moong dhal payasam.

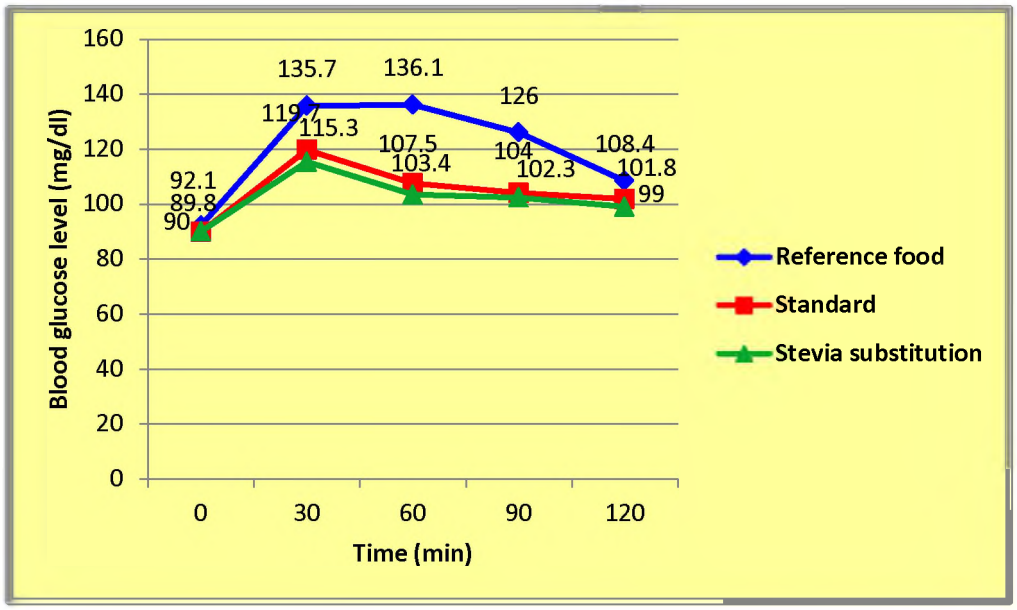


FIGURE 7

Glycemic response of rava kheer substituted with *Stevia rebaudiana* leaves

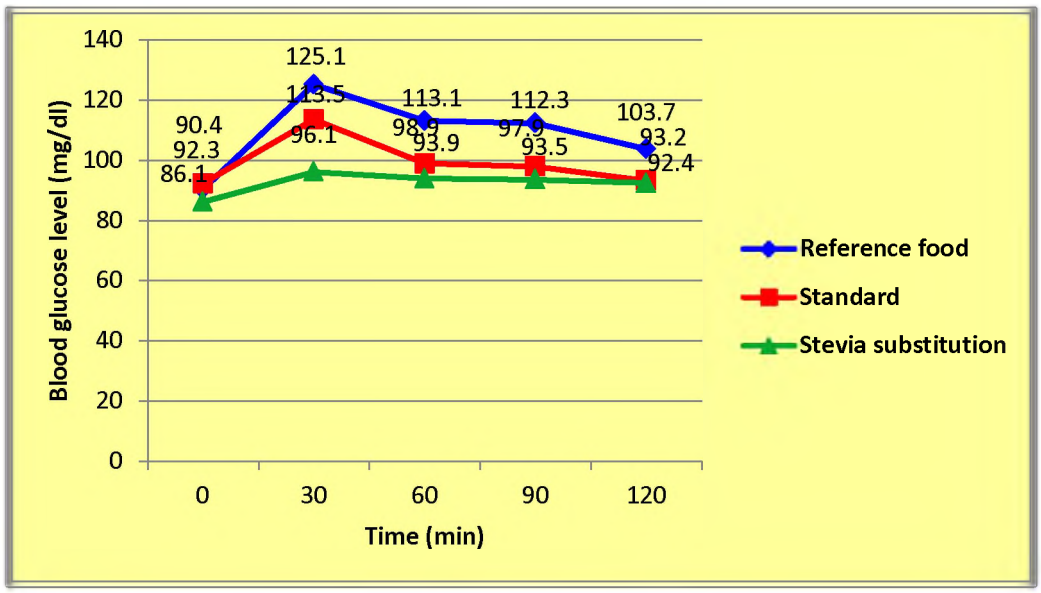


FIGURE 8

Glycemic response of moong dhal payasam substituted with *Stevia rebaudiana* leaves

Glycemic index of the desserts substituted with *Stevia rebaudiana* leaves

According to Tabassum *et al.,* (2013), glycemic index is a concept of ranking foods rich in carbohydrate according to blood glucose response after their consumption. Foods are classified as low, medium and high glycemic index of $\leq$ 55, 55-70 and $\geq$ 70 according to rate they influence on blood glucose levels. The glycemic index of the desserts substituted with *Stevia rebaudiana* leaves is revealed in Table XIII.

TABLE XIII

GLYCEMIC INDEX OF THE DESSERTS SUBSTITUTED WITH *STEVIA REBAUDIANA* LEAVES

Desserts	Glycemic index	
	Standard	*Stevia* substitution
Rava kheer	85.24	82.21
Moong dhal payasam	88.83	82.76

From the above table, the glycemic index of standard rava kheer and *Stevia rebaudiana* substituted rava kheer was 85.24 and 82.21 respectively. The glycemic index of *Stevia rebaudiana* substituted rava kheer was lower than standard rava kheer. The glycemic index of standard moong dhal payasam and *Stevia rebaudiana* substituted moong dhal payasam was 88.8 and 82.76 respectively. The glycemic index of *Stevia rebaudiana* substituted moong dhal payasam was lower than standard moong dhal payasam.

A study done by Arora and Jood (2016), with the traditional recipes like sweet porridge, carrot halwa and kheer incorporated with *Stevia rebaudiana* leaf powder and subjected to glycemic index study, wherein the glycemic index of the traditional recipes incorporated with *Stevia rebaudiana* leaf powder showed a low glycemic index than the standard traditional recipes.

Although the desserts substituted with *Stevia rebaudiana* leaves fall in the high glycemic index food category, they had a comparatively lower glycemic index than the standard desserts. The methods of cooking such as mashing or pureeing foods (Collier and O'Dea, 1982) causes disruption of the starch molecules making

the amylose and amylopectin molecules ready for hydrolysis greatly influencing the glycemic index (Pi-Sunyer, 2002).

The factors affecting the glycemic index like the form of food, preparation methods, processing of foods, macronutrient composition, heat utilized, and the amount of water and the time of cooking of the food tested for glycemic index may be the reason for the high glycemic index of the desserts substituted with *Stevia rebaudiana* leaves as the result.

V. SUMMARY AND CONCLUSION

Excessive intake of sugar is not good for a healthy lifestyle as it is devoid of nutritional benefits made of empty calories. Consumption of high quantities of fat and sugar, sugar sweetened snacks and beverages and leading a sedentary lifestyle is a major nutritional problem faced by mankind in this 21st century and is associated with serious health problems like diabetes and metabolic disorders like obesity. *Stevia rebaudiana* commonly known as sweet leaf or sugar leaf is an anti-diabetic sweetener herb and can sweeten a number of foods and beverages in the form of fresh or dried leaves or tincture.

The leaves of *Stevia rebaudiana* contain protein, fat, carbohydrates, vitamins like folic acid, vitamin C and minerals including potassium, calcium, magnesium, phosphorus, phytochemicals and all indispensable aminoacids except tryptophan. Having potent sweetness intensities and providing a cost effective sucrose substitute, *Stevia rebaudiana* leaves can change a person's health risk by a slight alteration in the diet alternative to sucrose.

The present study entitled "**Evaluation and development of beverages and desserts substituted with *Stevia rebaudiana* leaves**" was aimed at evaluation of proximate principles, phytochemicals, anti-microbial activity, toxic substances and developing diabetic friendly beverages and desserts substituted with *Stevia rebaudiana* leaves. The analysis of proximate principles of dried *Stevia rebaudiana* leaves was carried out that included moisture, protein, ash, fat, crude fibre, carbohydrate, starch, energy, ascorbic acid, beta carotene, calcium, iron and phosphorus. Qualitative analysis of phytochemical present in *Stevia rebaudiana* leaves such as tannins, terpenoids, phenols, saponins, quinones, glycosides, coumarins, sterols, flavonoids and alkaloids was conducted in aqueous and solvent extracts viz. ethanol and methanol.

The antimicrobial activity in the *Stevia rebaudiana* leaves was studied including two bacterial strains a gram negative bacteria (*Escherichia coli*) and a gram positive bacteria (*Staphylococcus aureus*) and two fungal strains namely *Aspergillus flavus* and *Aspergillus niger*. The antimicrobial activity was studied by measuring the Diameter of Zone of Inhibition (IZD).

The toxic substances such as aflatoxin and ochratoxin content were evaluated in the *Stevia rebaudiana* leaves. Diabetic friendly beverages like tea and coffee and desserts like rava kheer and moong dhal payasam substituted with *Stevia rebaudiana* leaves were developed after standardization of the beverages and desserts after repeated trials with different concentrations and methods of incorporation.

The developed beverages and desserts were subjected to sensory evaluation by 30 semi-trained panel members along with standard and three different variations (Variation I, II and III) with the help of score card (9 point hedonic scale). The evaluation was done in terms of the attributes like appearance, colour, flavour, texture, taste and overall acceptability of the recipes by rating from like extremely to dislike extremely in the 9 point hedonic scale rating.

The glycemic index was determined for the developed desserts with the help of study volunteers by measuring the blood glucose levels using a glucometer. The blood glucose levels were checked for every 30, 60, 90 and 120 min for the postprandial glucose level and the blood glucose response curve for the 2 hour period was used to determine the glycemic index of the developed desserts substituted with *Stevia rebaudiana* leaves. The study protocol was approved by the institutional Ethical Committee of Avinashilingam Institute for Home Science and Higher Education for Women and the approval number was AUW/ IHEC/ FSN -17-18/XPD/17. The sensory evaluation of the beverages and desserts were statistically analyzed for men and standard deviation to find the best acceptable one.

The salient findings of the study are summarized below:

- The moisture content of *Stevia rebaudiana* leaves was 3.53 % per 100 g of leaves. The protein content was 16.66 g, ash content with 10 g, fat content with 4.66 g and crude fibre with 11.58 g per 100 g of the leaves. The carbohydrate content of *Stevia rebaudiana* leaves was 32.50 g and starch present in 1.66 g per 100g of the leaves. The energy value of *Stevia rebaudiana* leaves per 100g was 238.66 kcal. Vitamins and minerals which were analyzed include ascorbic acid, beta carotene, calcium, iron and phosphorus. The ascorbic acid present in 100 g of *Stevia rebaudiana* leaves

was 30.95 mg. Beta carotene was in the level of 213.33 µg per 100 g of the leaves and the calcium content was found to be 726.66 mg. The iron content of *Stevia rebaudiana* leaves was11.93 mg and the phosphorus content of *Stevia rebaudiana* leaves was 386.66 mg per 100 g.

- The aqueous extract of *Stevia rebaudiana* leaves showed a strong presence of phytochemicals like terpenoids, saponins, glycosides, coumarins and sterols. Tannins, quinones, flavonoid and alkaloids were present in aqueous extract; while phenols were weakly present in aqueous extract. The ethanol extract of *Stevia rebaudiana* leaves indicated the strong presence of terpenoids, glycosides and sterols. The phytochemicals such as tannins, phenols, coumarin, flavonoids and alkaloids were weakly present in the ethanol extract of *Stevia rebaudiana* leaves. Saponins and quinones were absent in the ethanol extract of *Stevia rebaudiana* leaves. The methanol extract of *Stevia rebaudiana* leaves indicated a strong presence of terpenoids, glycosides and sterols. The methanol extract showed the presence of phytochemicals such as tannins, phenols, coumarins and flavonoids. The phytochemicals such as glycosides and flavonoids were weakly present in methanol extract of *Stevia rebaudiana* leaves whereas saponins was completely absent in methanol extract of *Stevia rebaudiana* leaves.
- The antibacterial activity of aqueous extract of *Stevia rebaudiana* leaves showed a zone of inhibition of 9.0 mm which was greater than that of control with 8.5 mm against *Escherichia coli*. The zone of inhibition was 11.0 mm against *Staphylococcus aureus* higher than that of the control with 9.5 mm in aqueous extract of *Stevia rebaudiana* leaves. The control *Aspergillus flavus* showed zone of inhibition of 6.0 mm whereas the aqueous extract of *Stevia rebaudiana* leaves showed a greater zone of inhibition of 11.5 mm than the control. The zone of inhibition of *Stevia rebaudiana* leaf extract against *Aspergillus flavus* was observed to be 13.0 mm which was significantly higher than the control with 7.0 mm. The results of this study indicate that the Stevia rebaudiana leaf extract have inhibitory activities against microorganisms, as they have significantly higher activity than the control.

- The *Stevia rebaudiana* leaves contained aflatoxin in the quantities of 9.0 ppb which is lesser than the action level. Ochratoxin was found to be below the detection limit. The results of this finding clearly shows that *Stevia rebaudiana* leaves are safe for consumption since the content of the toxins lie in the safe upper limit.
- Sensory evaluation of coffee substituted with *Stevia rebaudiana* leaves revealed that the appearance for standard was 8.86, variation I, 8.63, variation II, 8.20 and variation III was 7.70. The mean values obtained for colour was 8.83 for standard, 8.36 for variation I, 8.00 for variation II and 7.76 for variation III. The mean scores obtained for the flavour in coffee substituted with *Stevia rebaudiana* leaves ranged from 7.00 to 8.83, the maximum score obtained for standard with a mean value of 8.83 followed by variation I with 8.16, variation II with 7.73 and variation III with 7.00. With regard to consistency, standard showed the best result with a mean value of 8.86 followed by variation I with 8.26, variation II with 7.80 and variation III with 7.06. The taste of the standard scored maximum mean value of 8.83 followed by 7.80, 7.16 and 6.50 for variation I, variation II and variation III respectively. The overall acceptability of coffee substituted with *Stevia rebaudiana* leaves revealed that variation I had a maximum mean score of 7.93 and was comparable with the standard of 8.83. This was followed by variation II and II with mean scores 7.50 and 6.66 respectively. It was observed that the overall acceptability of coffee with *Stevia rebaudiana* leaf infusion was statistically significant at 5 % level when standard and variation I was compared. No significant change was observed for overall acceptability for variation I, II and III.
- The appearance of the tea substituted with *Stevia rebaudiana* leaves for standard, variations I, II and III, standard scored the best result with mean value of 8.96, variation III scoring a decreased score of 8.13. In terms of colour, standard and three variations got mean scores from 7.96 to 8.96. Variation I and II had a mean value of 8.73 and 8.53 respectively. The maximum mean score for colour was for standard with 8.96. The mean scores obtained for flavor ranged from 7.10 to 8.90, the minimum score 7.10 for variation III, 8.03 for variation II and 8.06 for variation I and 8.90 for standard. Regarding consistency, mean scores of standard showed a value

of 8.93, variation I and variation II with 8.36 each and variation III with 7.63. Taste contributed mean scores from 6.80 to 8.93.The maximum mean value obtained for standard was 8.93 followed by variation I with 7.96, variation II with 7.90 and variation III with 6.80. The mean overall acceptability score was 8.96 for standard; 8.10 for variation I; 8.00 for variation II and 7.00 for variation III respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III. It was observed that the overall acceptability was not significant when standard and variations were compared.

- The appearance of standard was maximum with mean value of 8.36 followed by variation I with 8.10, variation II with 8.03 and variation III with 7.46 for rava kheer substituted with *Stevia rebaudiana* leaves. Colour contributed mean scores from 7.26 to 8.56. The maximum mean score for colour obtained for standard with 8.56 followed by 8.13, 8.03 and 7.26 for variation I, II and III respectively. In terms of flavour and texture, standard got mean value of 8.46 and 8.26 respectively, variation I got 7.33 and 7.76 respectively, variation II got 7.16 and 7.83 respectively and variation III got 5.73 and 7.16 respectively. The taste of the standard, variation I, variation II and variation III secured a mean value of 8.56, 7.16, 6.70 and 5.10 correspondingly. Overall acceptability of standard was 8.56, variation I was 7.20, variation II was 7.10 and variation III was 5.53 respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III. The F values were 2.510, 2.302 and 0.557 for variation I, II and III respectively. The results implied that the variations were not significant statistically.
- The results of sensory evaluation of moong dhal payasam substituted with *Stevia rebaudiana* leaves revealed that in terms of appearance, standard scored best with a mean score of 8.83 followed by variation I with 8.66, variation II with 8.63 and variation III with 8.56. In terms of colour, variation II had minimum score of 8.53 followed by variation III with 8.56, variation I with 8.63 and standard with 8.90. The mean scores obtained for the flavour in moong dhal payasam substituted with *Stevia rebaudiana* leaves ranged from 5.80 to 8.73, the maximum obtained by standard with a mean of 8.73 followed by 7.80, 7.26 and 5.80 for variation I, II and III respectively.

Regarding texture, mean scores of standard showed a maximum value of 8.66 followed by variation II with 8.46, variation I with 8.43 and variation III with 8.23 respectively. Taste is an important attribute. Taste contributes scores from 5.46 to 8.86 and maximum being in standard with 8.86 followed by variation I with 7.76, variation II with 7.03 and variation III with 5.46. In terms of overall acceptability, standard obtained 8.93 followed by variation I with 7.90 mean score; variation II with 7.40 and variation III with 5.56 respectively. The overall acceptability was analyzed statistically using F test comparing standards with variations I, II and III. The F values were 0.079, 2.287 and 0.098 for variation I, II and III respectively. The results revealed that the variations are not significant statistically.

- The overall acceptability of beverages substituted with *Stevia rebaudiana* leaves showed that, for coffee standard scored a mean maximum value with 8.83 followed by variation I with 7.93, variation II with 7.50 and variation III with 6.66. For tea, the maximum overall acceptability secured for standard with mean value of 8.96, variation I with 8.10, variation II with 8.00 and variation III with 7.00. The overall results indicate that variation I with 5 ml *Stevia rebaudiana* leaf infusion instead of sugar had an overall acceptability when compared with variation II and variation III. Hence this level of substitution can be included in the beverages which may enhance the nutritional value of the beverage consumed.
- The overall acceptability of desserts substituted with *Stevia rebaudiana* leaves showed that, for rava kheer, a score of 8.56 for standard with mean maximum score followed by variation I with 7.20, variation II with 7.10 and variation III with 5.53. The overall acceptability of moong dhal payasam secured 8.93 for standard, 7.90 for variation I, 7.40 for variation II and 5.56 for variation III. The overall results indicate that variation I with 7.5 ml *Stevia rebaudiana* leaf infusion for sugar was best acceptable when compared with variation II and variation III. Hence this level of substitution can be included in the desserts. This may enhance the nutritional value of the desserts with a guilt free consumption of desserts for diabetics and calorie conscious consumers.
- Rava kheer- The mean glycemic response of the reference food was found to be 92.1, 135.7, 136.1, 126.0 and 108.4 mg/dl at fasting, 30, 60, 90 and

120 minutes respectively. The mean glycemic response of rava kheer prepared with sugar (standard) was 89.8, 119.7, 107.5, 104.0 and 101.8 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively and the mean glycemic response of rava kheer substituted with *Stevia rebaudiana* leaves in the form of infusion was 90.0, 115.3, 103.4, 102.3 and 99.0 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The peak value was 136.1 mg/dl for reference food observed at 60 min whereas the peak value was 119.7 and 115.3 mg/dl for standard rava kheer and *Stevia rebaudiana* substituted rava kheer at 30 min. Thereafter the levels showed a decreasing trend at 120 min. White bread recorded the highest response (108.4 mg/dl) followed by standard rava kheer (101.8 mg/dl) and the lowest response (99.0 mg/dl) by *Stevia rebaudiana* substituted rava kheer.

- Moong dhal payasam- The mean glycemic response of the reference food was found to be 90.4, 125.1, 113.1, 112.3 and 103.7 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The mean glycemic response of moong dhal payasam prepared with sugar (standard) was 92.3, 113.5, 98.9, 97.9 and 93.2 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively and the mean glycemic response of moong dhal payasam substituted with *Stevia rebaudiana* leaves in the form of infusion was 86.1, 96.1, 93.9, 93.5 and 92.4 mg/dl at fasting, 30, 60, 90 and 120 minutes respectively. The peak value was 125.1 mg/dl for reference food observed at 30 min whereas the peak value was 113.5 and 96.1 mg/dl for standard moong dhal payasam and *Stevia rebaudiana* substituted moong dhal payasam at 30 min respectively. Thereafter the levels showed a decreasing trend at 120 min for reference food and standard moong dhal payasam. White bread recorded the highest response (103.7 mg/dl) followed by standard moong dhal payasam (93.2 mg/dl) and the lowest response (92.4 mg/dl) by *Stevia rebaudiana* substituted moong dhal payasam.
- The glycemic index of standard rava kheer and *Stevia rebaudiana* substituted rava kheer was 85.24 and 82.21 respectively. The glycemic index of *Stevia rebaudiana* substituted rava kheer was lower than standard rava kheer. The glycemic index of standard moong dhal payasam and *Stevia rebaudiana* substituted moong dhal payasam was 88.8 and 82.76 respectively. The glycemic index of *Stevia rebaudiana* substituted moong

dhal payasam was lower than standard moong dhal payasam. Although the desserts substituted with *Stevia rebaudiana* leaves fall in the high glycemic index food category, they had a comparatively lower glycemic index than the standard desserts.

It can be concluded from the present study that, *Stevia rebaudiana* a sweet herb is rich in nutrients such as protein, carbohydrate, calcium, phosphorus and beta carotene. It is also rich in phytochemicals like terpenoids, glycosides, sterols, tannins, quinones, coumarins, flavonoids, alkaloids and phenols which possess various nutraceutical properties. The aqueous extract of *Stevia rebaudiana* leaves possess very good anti-microbial activity and the level of toxic substances such as aflatoxin and ochratoxin were very low. The substitution of sugar with *Stevia rebaudiana* leaf infusion in various desserts showed a lower glycemic index when compared with standard desserts. Hence it can be concluded that *Stevia rebaudiana* leaf infusion is a safe and effective substitute for sugar and is a boon for diabetics in the present day scenario. It is the best alternative to sugar with phytochemicals and antimicrobial properties which is safe for consumption.

Recommendations for further research:

- ✓ Development of diabetic friendly recipes by substitution of *Stevia rebaudiana* leaves in traditional sweets, ice-creams, desserts, bakery products, etc.
- ✓ Carrying out studies on glycemic index by substituting *Stevia rebaudiana* in other recipes
- ✓ Bringing out the potentials of *Stevia rebaudiana* in other diseases
- ✓ Identification of other diabetic friendly herbs
- ✓ Development and standardization of recipes using diabetic friendly herbs
- ✓ Utilization of underutilized or extincting sweetener herbs in food products

BIBLIOGRAPHY

1. Abdo, B.M. and Genet, W. (2016). Sweetness equivalence ratio of *Stevia (Stevia Rebaudiana* Bertoni) with sugar adopted in Ethiopia; *International Journal of Advanced Biological and Biomedical Research*; Article 7, Volume 4, Issue 1, March 2016, Page 58-61.
2. Adari, B.R., Alavala, S., George, S.A., Meshram, H.M., Tiwari, A.K. and Sarma, A.V.S. (2016). Synthesis of rebaudioside-A by enzymatic transglycosylation of stevioside present in the leaves of *Stevia rebaudiana* Bertoni. *Food Chemistry*, Volume 200, 1 June 2016, Pages 154-158.
3. Ameer, K., Chun, B. and Kwon, J. (2017). Optimization of supercritical fluid extraction of steviol glycosides and total phenolic content from *Stevia rebaudiana* (Bertoni) leaves using response surface methodology and artificial neural network modeling. *Industrial Crops and Products*, Volume 109, 15 December 2017, Pages 672-685.
4. Andersen, B.V., Mielby, L.H., Viemose, I., Bredie, W.L.P. and Hlydig, G. (2017). Integration of the sensory experience and post-ingestive measures for understanding food satisfaction. A case study on sucrose replacement by *Stevia rebaudiana* and addition of beta glucan in fruit drinks. *Food Quality and Preference,* Volume 58, June 2017, Pages 76-84.
5. Andrae-Marobela, K., Ghislain, F.W., Okatch, H. and Majinda, R. (2013). Polyphenols: a diverse class of multi-target anti-HIV-1agents. *Current Drug Metabolism* 7:392.
6. Anton, S.D., Martin, C.K., Han, H., Coulon, S., Cefalu, W.T., Geiselman, P. and Williamson, D.A, (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite*. Aug; 55(1): 37–43.
7. AOAC. (1990): Association of official, chemists, official methods of analysis. 15th Edition, Washington DC, U.S.A.
8. AOAC. (2005). Determination of Moisture, Ash, Protein and Fat. *Official method of analysis of the association of analytical chemists*, 18th Ed. Washington DC.
9. Areli, C.L., Yolanda, M.O. and Maira, S.C. (2017). *Stevia rebaudiana* Bertoni: A Natural Alternative for Treating Diseases Associated with

Metabolic Syndrome. *Journal of Medicinal Food*. October 2017, 20(10): 933-943.

10. Arora, S. and Jood, S, (2016). Impact of Stevia incorporated buns on glycaemic response in normal, obese and diabetic subjects. *International Journal of Applied Home Science*. Volume 3 (5&6), May & June (2016): 214-220.
11. Arriola, N.D.A., Medeiros, P.M., Prudencio, E.S., Muller, C.M.O., Mello, R.D. and Amboni, C. (2016). Encapsulation of aqueous leaf extract of *Stevia rebaudiana* Bertoni with sodium alginate and its impact on phenolic content. *Food Bioscience*, Volume 13, 1 March 2016, Pages 32-40.
12. Ashwell, M. (2015). *Stevia*, Nature's Zero-Calorie Sustainable Sweetener, A New Player in the Fight against Obesity. *Nutrition Today*. 2015 May; 50(3): 129–134.
13. Balaswamy, K., Rao, P.P., Rao, G.N., Nagender, A. and Satyanarayana, A, (2014).
    Production of Low Calorie Ready-to-Serve Fruit Beverages Using a Natural Sweetener*, Stevia (Stevia Rebaudiana). Focusing on Modern Food Industry (FMFI)* Volume 3.
14. Barba, F.J., Criado, M.N., Belda-Galbis, C.M., Esteve, M.J. and Rodrigo, D. (2014). *Stevia rebaudiana* Bertoni as a natural antioxidant/antimicrobial for high pressure processed fruit extract: Processing parameter optimization. *Food Chemistry*, Volume 148, 1 April 2014, Pages 261-267.
15. Barroso, M.R., Martins, N., Barros, L., Antonio, A.L., Rodrigues, A., Sousa, M.J., Santos-Buelga, C. and Ferreira, I.C.F.R (2018). Assessment of the nitrogen fertilization effect on bioactive compounds of frozen fresh and dried samples of *Stevia rebaudiana* Bertoni. *Food Chemistry*, Volume 243, 15 March 2018, Pages 208-213.
16. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology.* 1966 Apr; 45(4):493–496.
17. Brandle, J. E., Starratt, A. N. and Gijzen, M. (1998). *Stevia rebaudiana*: Its agricultural, biological, and chemical properties. *Canadian Journal of Plant Science*. 78, 527–536.

18. Capella, J.M.C., Buniowska, M., Cortes, C., Zulueta, A., Frigola, A. and Esteve, M.J. (2017). Influence of pulsed electric field processing on the quality of fruit juice beverages sweetened with *Stevia rebaudiana*. *Food and Bioproducts Processing*, Volume 101, January 2017, Pages 214-222.
19. Chan, P., Tomlinson, B., Chen, Y.-J., Liu, J.-C., Hsieh, M.-H. and Cheng, J.-T. (2000). A double-blind placebo-controlled study pf the effectiveness and tolerability of oral stevioside in human hypertension. *British Journal of Clinical Pharmacology,* 50, 215–220.
20. Chen, T., Chen, S., Chan, P. and Chu, Y. (2005). Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. *Plant Medicine*, 71: 108-213.
21. Choudhary, S.K., Singh, S., Singh, R.K., Upadhyay, P.K. and Singh, A., (2014). *Stevia rebaudiana* Bertoni: An Alternative Option of Sugar Crop in India. *Popular Kheti,* Volume 2, Issue 4.
22. Chouhan, E.S., Srivastava, S. and Chawla, P. (2016). Proximate and phytochemical analysis of *Stevia* leaves powder. *International Ayurvedic Medical Journal*: Volume 4; Issue 08; August 2016.
23. Collier, G. and O'Dea, K. (1982). Effect of physical form of carbohydrate on the postprandial glucose, insulin and gastric inhibitory polypeptide responses in Type 2 Diabetes. *The American Journal of Clinical Nutrition*; 36:10–4.
24. Debnath, M. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana. Journal of Medicinal Plants Research* Vol. 2(2), pp. 045-051.
25. Dodd, H., Williams, S., Brown, R. and Venn, B. (2011). Calculating meal glycemic index by using measured and published food values compared with directly measured meal glycemic index. *The American Journal of Clinical Nutrition*, Volume 94, Issue 4, 1 October 2011, Pages 992–996.
26. Doughari, J.H., Ndakidemi, P.A., Human, I.S. and Benade, S. (2012). Antioxidant, antimicrobial and antiverotoxic potentials of extracts of Curtisia dentata. *Journal of Ethnopharmacology*, 141:1041–1050.
27. Drewnowski, A. and Moskow, H. (1985). Sensory characteristics of foods: new evaluation techniques. *The American Journal of Clinical Nutrition* 42: pp 924-931.

28. Entwisle, A.C., Williams, A.C., Mann, P.J., Slack, P.T. and Gilbert, J. (2000). Liquid chromatographic method with immunoaffinity column cleanup for determination of ochratoxin A in barley: collaborative study. *Journal of AOAC International*; 83:1377–1383.
29. FSSAI (2012). Manual of Methods of Analysis of Foods Mycotoxins, Lab Manual 7.
30. Gardana, C., Scaglianti, M. and Simonetti, P. (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high performance liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 217:1463–1470.
31. Genus, J.M.C. (2007). *Stevia rebaudiana* Bertoni plants and dried leaves as Novel Food. *Eurpoean Stevia Association.*
32. Ghanta, S., Banerjee, A., Poddar, A. and Chattopadhyay, S. (2007). Oxidative DNA Damage Preventive Activity and Antioxidant Potential of *Stevia rebaudiana* (Bertoni) Bertoni, a Natural Sweetener. *Journal of Agriculture and Food Chemistry*, 2007, 55 (26), pp 10962–10967.
33. Ghosh, S., Subudhi, E. and Nayak, S. (2008). Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *International Journal of Integrative Biology*, No. 1, 27-31.
34. Goyal, S., Samsher, and Goyal, R. (2010). *Stevia* (*Stevia rebaudiana*) a bio-sweetener: a review. *International Journal of Food Sciences and Nutrition,* 61:1-10.
35. Guggisberg, D., Piccinali, P. and Schreier, K, (2011). Effects of sugar substitution with *Stevia*, Actilight and *Stevia* combinations or Palatinose on rheological and sensory characteristics of low-fat and whole milk set yoghurt. *International Dairy Journal*, Volume 21, Issue 9, September 2011, Pages 636-644.
36. Gulati, S. and Misra, A., (2014). Sugar Intake, Obesity, and Diabetes in India. *Nutrients*, 6, 5955-5974.
37. Gupta, E., Purwar, S., Sundaram, S. and Rai, G.K. (2013). Nutritional and therapeutic values of *Stevia rebaudiana:* A review. *Journal of Medicinal Plants Research*, Vol. 7(46), pp. 3343-3353.
38. Hanspal, S. (2010). Consumer Survey on Sustainable Tea & Coffee Consumption, Published By Partners in Change. 2010.

39. Hracek, V. M., Gliemmo, M. F. and Campos, C. A. (2010). Effect of steviosides and system composition on stability and antimicrobial action of sorbates in acidified model aqueous systems. *Food Research International*; 43: 2171-2175.

40. Huang, X.Y., Jun-FangFu and Duo-LongDi (2010). Preparative isolation and purification of steviol glycosides from *Stevia rebaudiana* Bertoni using high-speed counter-current chromatography. *Separation and Purification Technology*, Volume 71, Issue 2, 18 February 2010, Pages 220-224.

41. Jae-Yong K., Juyi, S. and Kyung-Hyun C. (2011). Aspartame-fed zebra fish exhibit acute deaths with swimming defects and saccharin-fed zebra fish have elevation of cholesteryl ester transfer protein activity in hypercholesterolemia. *Food and Chemical Toxicology* 49 (11): 2899-2905.

42. Jain, J.L., Jain, S. and Jain, N (2007). *Fundamentals of biochemistry;* New Delhi: S. Chand & Co. Pub. Ltd. pp. 104–107.

43. Javed, R., Usman, M., Yucesan, B., Zia, M. and Gurel, E. (2017). Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of *Stevia rebaudiana* Bertoni. *Plant Physiology and Biochemistry*, Volume 110, January 2017, Pages 94-99.

44. Jayaraman, S., Manoharan, M. and Illanchezian, S. (2008). In-vitro anti-microbial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. *Tropical Journal of Pharmaceutical Research.* 7:1143–1149.

45. Jenkins, D.J.A., Wolever, T.M.S., Jewkins, A.L. and Josse, R.G. (1981). The Glycemic Index: Methodology and Clinical Implication. *The American Journal of Clinical Nutrition*, Vol. 54, pp. 846-854.

46. Jentzsch, P.V., Torrico-Vallejos, S., Mendieta-Brito, S., Ramos, L.A. and Ciobota, L. (2016). Detection of counterfeit *Stevia* products using a handheld Raman spectrometer. *Vibrational Spectroscopy*, Volume 83, March 2016, Pages 126-131.

47. Jeppesen, P.B., Gregersen, S. and Poulsen, C.R. (2000). Stevioside acts directly on pancreatic beta cells to secrete insulin: Actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K^+-channel activity. *Metabolism* 49(2):208–214.

48. Jitendra, M., Monika, S., Ratan, S.D., Priyanka, G., Priyanka, S. and Kiran, D.J. (2012). Micropropagation of an Anti-diabetic Plant - *Stevia rebaudiana*

Bertoni, (Natural Sweetener) in Hadoti Region of South-East Rajasthan, India. ISCA Journal of Biological Sciences 1(3), 37-42.

49. Kafle, G.G., Midmore, D.J. and Gautam, R. (2017). Effect of nutrient omission and pH on the biomass and concentration and content of steviol glycosides in *Stevia* (*Stevia rebaudiana* (Bertoni) Bertoni) under hydroponic conditions. *Journal of Applied Research on Medicinal and Aromatic Plants*, Volume 7, December 2017, Pages 136-142.
50. Kalpana, M., Anbazhagan, M., Natarajan, V. and Dhanavel, D. (2010). Improved micropropagation method for the enhancement of biomass in *Stevia rebaudiana* Bertoni. *Recent Research in Science and Technology* 2(1): 008–013.
51. Kaushik, R., Narayanan, P., Vasudevan, V., Muthukumaran, G. and Usha, A. (2010). Nutrient composition of cultivated *Stevia* leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. *Journal of Food Science and Technology*, January 2010, Volume 47, Issue 1, pp 27–33.
52. Kim, J., Choi, Y.H. and Choi, Y.H. (2011). Use of stevioside and cultivation of *Stevia rebaudiana* in Korea. In: Kinghorn, A.D. (Ed.), *Stevia*, the Genus *Stevia*. *Medicinal and Aromatic Plants-Industrial Profiles*, Taylor and Francis, London and NY. 19:196–202.
53. Kroyer, G (2010). Stevioside and *Stevia*-sweetener in food: application, stability and interaction with food ingredients. *Journal of Consumer Protection and Food Safety*, May 2010, Volume 5, Issue 2, pp 225–229.
54. Kujur, R.S., Singh, V., Ram, M., Yadava, N., Singh, K.K., Kumari, S. and Roy, B.K. (2010). Antidiabetic Activity and Phytochemical Screening of Crude Extract of *Stevia Rebaudiana* in Alloxan-induced Diabetics Rats. *Pharmacognosy Journal*, Volume 2, Issue 14, September 2010, Pages 27-32.
55. Kumar, H., Kaul, K., Bajpai-Gupta, S., Kaul, V.K. and Kumar, S. (2012). A comprehensive analysis of fifteen genes of steviol glycosides biosynthesis pathway in *Stevia rebaudiana* (Bertoni). *Gene*. 2012 Jan 15; 492(1):276-84.
56. Langle, A., Gonzalez-Coronel, M.A., Carmona-Gutierrez, G., Moreno-Rodríguez, J.A., Venegas, B., Munoz, G., Trevino, S. and Diaz, A, (2015). *Stevia rebaudiana* loaded titanium oxide nanomaterials as an antidiabetic

agent in rats. *Revista Brasileira de Farmacognosia*, Volume 25, Issue 2, March–April 2015, Pages 145-151.

57. Latha, S., Chaudhary, S. and Ray, R.S. (2017). Hydroalcoholic extract of *Stevia rebaudiana* bert. leaves and stevioside ameliorates lipopolysaccharide induced acute liver injury in rats. *Biomedicine & Pharmacotherapy*, Volume 95, November 2017, Pages 1040-1050.
58. Lemus-Mondaca, R., Vega-Galvez, A., Zura-Bravo, L. and Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*, Volume 132, Issue 3, 1 June 2012, Pages 1121-1132.
59. Lustig, R.H., Schmidt, L.A. and Brindis, C.D. (2012). Public Health: the toxic truth about sugar. *Nature;* 482(7383), 27.
60. Lyakhoukin, A.G., Long, D.A. and Titoy, M.P. (1993). Cultivation and utilization of S*tevia (Stevia rebaudiana* Bertoni) Agricultural Publishing House Hanoi, Vietnam 1 44.
61. Maki, K.C., Curry, L. L. and Carakostas, M. C. (2008). The hemodynamic effects of rebaudioside A in healthy adults with normal and low normal blood pressure. *Food Chemistry Toxicology*, 46: 40-46.
62. Manay, S.N., and Shadaksharaswamy, M. (2007). *Food Facts and Principles*, New Age International. (P) Ltd Publishers pp- 220-232.
63. Matsui, K., Kawasaki, Y., Oda, Y., Noguchi, T., Kitagaewa, Y. and Sawada, M. (1996). Evaluation of the genotoxicity of stevioside and steviol using six in vitro and one in vivo mutagenicity assays. *Mutagenesis,* 11: 573-579.
64. Mazumder, P.M. and Sasmal, D. (2001). Mycotoxins – Limits and Regulations. *Ancient Science Life.* Jan-Mar; 20(3): 1–19.
65. Megeji, N.W., Kumar, J.K., Singh, V., Kaul, V.K. and Ahuja, P.S. (2005). Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. *Current Science*. 88:801-804.
66. Midmore, D.J. and Rank, A.H. (2002). A new rural industry *Stevia* to replace imported chemical sweeteners. *A report for the Rural Industries Research and Development Corporation*, August 2002.

67. Mishra, N. (2011). An Analysis of antidiabetic activity of *Stevia rebaudiana* extract on diabetic patient. *Journal of Natural Sciences Research,* Vol.1, No.3.

68. Mishra, P., Singh, R., Kumar, U. and Prakash, V. (2010). *Stevia rebaudiana*– A magical sweetener. *Global Journal of Biotechnology and Biochemistry*. 5:62–74.

69. Mohd-Radzman, N. H., Ismail, W. I. W., Jaapar, S. S., Adam, Z. and Adam, A. (2013). Stevioside from *Stevia rebaudiana* Bertoni increases insulin sensitivity in 3T3-L1 adipocytes. *Evidence-Based Complementary and Alternative Medicine*, 2013, 1–9.

70. Muanda, F.N., Soulimani, R., Diop, D. and Dicko, A, (2011). Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana* Bertoni leaves. *LWT - Food Science and Technology*, Volume 44, Issue 9, November 2011, Pages 1865-1872.

71. Oliveira, F.I.P., Rodrigues, S. and Fernandes, F.A.N. (2012). Production of low calorie Malay apples by dual stage sugar substitution with *Stevia*-based sweetener. *Food and Bioproducts Processing*, Volume 90, Issue 4, October 2012, Pages 713-718.

72. Pacifico, S., Piccolella, S., Nocera, P., Tranquillo, E., Polggetto, F.D. and Catauro, M. (2017). Steviol glycosides content in cultivated *Stevia rebaudiana* Bertoni: A new sweet expectation from the Campania region (Italy). *Journal of Food Composition and Analysis*, Volume 63, October 2017, Pages 111-120.

73. Panagiotou, C., Mihailidou, C., Brauhli, G., Katsarou, O. and Moutsatsou, P. (2018). Effect of steviol, steviol glycosides and *Stevia* extract on glucocorticoid receptor signaling in normal and cancer blood cells. *Molecular and Cellular Endocrinology*, Volume 460, 15 January 2018, Pages 189-199.

74. Pandiyan, R., Velu, R.K., Chandrasekaran, S.K. and Bashiyam, P. (2009). Effect on extracts of *Stevia rebaudiana* bertoni in ethanol induced gastric ulcer by using wister rats. *Recent Research in Science and Technology*, 1(3):
127–130.

75. Paulraj, S., Rajasekaran, S. and Mathew, A.C. (2017). Prevalence of Depression and its Associated Factors in Adults with Diabetes Mellitus:

Across-Sectional Study in a Tertiary Care Centre. *Advanced Medical Sciences: An International Journal (AMS),* Vol 4, No.1/2/3.

76. Pi-Sunyer, F.X., (2002). Glycemic index and disease. *The American Journal of Clinical Nutrition.* 76 (suppl): 290S-8S.
77. Prakash, D., Gupta, C. and Sharma, G., (2012). Importance of Phytochemicals in Nutraceuticals. *Journal of Chinese Medicine Research and Development (JCMRD)* Oct. 2012, Vol. 1 Iss. 3, PP. 70-78.
78. Preethi, D., Sridhar, T.M. and Naidu, C.V. (2011). Carbohydrate Concentration Influences on In Vitro Plant Regeneration in *Stevia rebaudiana*, *Journal of Phytology*, 3, 61-64.
79. Raina, R., Bhandari, S.K., Chand, R. and Sharma, Y (2013). Strategies to improve poor seed germination in *Stevia rebaudiana*, a low calorie sweetener. *Journal of Medicinal Plants Research*, Vol. 7(24), pp. 1793-1799.
80. Ramesh, K., Singh, V. and Megeji, N.W. (2006). Cultivation of *Stevia* [*Stevia rebaudiana* (Bert.) Bertoni]: A Comprehensive Review. Advances in Agronomy, Volume 89, 2006, Pages 137-177.
81. Ramos-Tovar, E. and Muriel, P. (2017). *Stevia* as a Putative Hepatoprotector. *Liver Pathophysiology*, Pages 715-727.
82. Ranjan, R., Jaiswal, J. and Jena, J. (2011). *Stevia* as a natural sweetener. *International Journal of Research in Pharmacy and Chemistry,* 1(4).
83. Ranjan, R., Pradhan, Shweta, R., Sasmita, R., Bandita, D. and Shailja, R. (2014). Evaluation of post-harvest quality for aflatoxin and microbial loads on the leaves of *Stevia rebaudiana* Bertoni cultivated in Odisha. *International Journal of Innovation and Applied Studies*, vol. 9, no. 2, pp. 835–840.
84. Rieck, W.U. (2012). The leaves of *Stevia rebaudiana* (Bertoni), their constituents and the analyses thereof: a review. *Journal of Agricultural and Food Chemistry*; 60(4):886-95.
85. Robinson, R.L, (1930). Contributions from the Gray Herbarium of Harvard University XC. *The Gray Herbarium of Harvard University Cambridge*, MA 78-91.
86. Samsudin, A. and Aziz, I.A. (2013). Drying of *Stevia leaves* using laboratory and pilot scale dryers. *Journal of Tropical Agriculture and Food Science.* 41(1): 137– 147.

87. Santini, A., Ferrara, L., Naviglio, D., Aragon, A. and Ritieni, A. (2008). *Stevia*: non-caloric natural sweetener. *Progress in Nutrition,* 10, 3–10.

88. Sekaran, T., Giridhar, P. and Ravishankar, G.A., (2007). Production of steviosides in ex vitro and in vitro grown *Stevia rebaudiana* Bertoni. *Journal of the Science of Food and Agriculture,* 87, 420-424.

89. Serio, L. (2010). La *Stevia rebaudiana*, une alternative au sucre. *Phytothérapie*. 8:26–32.

90. Sethi, R., Smith, D. and Park, C., (2001). Cross-functional product development teams, creativity and innovativeness of new consumer products. *Journal of Marketing Research,* 38 (1), 73-86.

91. Shah, A.B., Jones, G.P. and Vasiljevic, T. (2010). Sucrose-free chocolate sweetened with *Stevia rebaudiana* extract and containing different bulking agents – effects on physicochemical and sensory properties. *International Journal of Food Science and Technology*, Volume 45, Issue 7 July 2010 Pages 1426–1435.

92. Shah, S.M.M. and Shah, S.M.H. (2015). Phytochemicals, antioxidant, antinociceptive and anti-inflammatory potential of the aqueous extract of Teucrium stocksianum bioss. *BMC Complementary and Alternative Medicine*; 15: 351.

93. Sharma, R., Yadav, R. and Manivannan, E. (2012). Study of effect of *Stevia rebaudiana* bertoni on oxidative stress in Type-2 Diabetic rat models. *Biomedicine & Aging Pathology*, Volume 2, Issue 3, July–September 2012, Pages 126-131.

94. Sharma, V.B. (2006). *A text book of food science and technology*. International book distributing co., Lucknow. Pp. 56.

95. Sheeja, R.R., and Lawrence, B. (2015). Phytochemical Screening of the Leaves of *Stevia rebaudiana*, Bertoni. *International Journal of Current Microbiology and Applied Sciences*, Volume 4 Number 3 (2015) pp. 344-347.

96. Shivanna, N., Naika, M., Khanum, F. and Kaul, V.K. (2013) Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. *Journal of Diabetes and its Complications.* 27, 103–113.

97. Shukla, S., Mehta, A., Bajpai, A.K. and Shukla, S. (2009). In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. *Food and Chemical Toxicology*, 47, 2338–2343.

98. Shukla, S.K., Kumar, R. and Dikshit, A. (2017). Bio-Sweetener (*Stevia rebaudiana* Bertoni): Boon for Rural India. *Organic and Medicinal Chemistry International Journal*; Volume 3 Issue 3 - September 2017.

99. Sichani, M.M., Karbasizadeh, V., Aghai, F. and Mofid, M.R. (2012). Effect of different extracts of *Stevia rebaudiana* leaves on Streptococcus mutans growth. *Journal of Medicinal Plants Research*, Vol. 5 No.32.

100. Siddique, A.B., Rahman, S.M.M. and Hossain, M.A. (2016). Chemical composition of essential oil by different extraction methods and fatty acid analysis of the leaves of *Stevia Rebaudiana* Bertoni. *Arabian Journal of Chemistry*. Volume 9, Supplement 2, November 2016, Pages S1185-S1189.

101. Sidel, J.L. and Stone, H. (1993). The role of sensory evaluation in the food industry. *Food Quality & Preference*, 4, 65-73.

102. Singh, S., Garg, V., Yadav, D., Beg, M.N. and Sharma, N. (2012). In vitro antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (Bertoni). *International Journal of Pharmacy and Pharmaceutical Sciences,* 4:468-473.

103. Sivaram, L. and Mukundan, U. (2003). In vitroculture studies on *Stevia rebaudiana*. *In Vitro Cellular & Developmental Biology*, 39(5): 520-523.

104. Soejarto, D.D., Compadre, C.M., Medon, P.J., Kamath, S.K. and Kinghorn, A.D. (1983). Potential sweetening agents of plant origin. II. Field search for sweet-tasting *Stevia* species. *Economic Botany*, 37: 71-79.

105. Soetan, K.O., Olaiya, C.O. and Oyewole, O.E., (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science Vol. 4(5) pp. 200-222, May 2010.

106. Soufi, S., D'Urso, G., Pizza, C., Rezgui, S., Bettaieb, T. and Montoro, P. (2016). Steviol glycosides targeted analysis in leaves of *Stevia rebaudiana* (Bertoni) from plants cultivated under chilling stress conditions. *Food Chemistry*, Volume 190, 1 January 2016, Pages 572-580.

107. Srilakshmi, B. (2014). Dietetics, Seventh Multi colour edition. New Age International Publisher, pp. 320.

108. Stahl, W and Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta*, Vol.1740, Pp.101–108.
109. Stephen, P.G. and Mark, E.C. (2011). Diabetic nephropathy in 2010: Alleviating the burden of diabetic nephropathy. *Nature Reviews Nephrology* **7**, 71–73.
110. Sukkar, M.Y. (1985). Human Nutrition for Medical studies and Applied Health Science. I Edition. Thaca, Press 13 South Work Street London.
111. Suttajit, M., Vinitketkaumnuen, U. and Meevatee, U. (1993). Mutagenicity and human chromosomal effect of stevioside, a sweetener from *Stevia rebaudiana* Bertoni. *Environmental Health Perspectives* 101(3):53–56.
112. Tabassum, F., Khan, A., Alam, I., Niamatullah, Khan, S., Khan, I., Zubair, M., Zafar, M., Qureshi, K., Safdar, M. and Din, Z. (2013). Determination of glycemic indices and glycemic loads of various types of cereal foods. *ARPN Journal of Agricultural and Biological Science*, Vol. 8, No. 6, June 2013.
113. Tadhani, M. and Subhash, R. (2006). Preliminary studies on *Stevia rebaudiana* leaves proximal composition, mineral analyses and phytochemical screening. *Journal of Medicinal Science*. 2006; 6(3):321-326.
114. Tavarini, S. and Angelini, L.G. (2013). *Stevia rebaudiana* Bertoni as a source of bioactive compounds: the effect of harvest time, experimental site and crop age on steviol glycoside content and antioxidant properties. *Journal of the Science of Food and Agriculture,* 93:2121–2129.
115. Tejo, V.K., Karsodihardjo, S. and Ananingsih, V.K. (2013). *Stevia Rebaudiana*: an Excellent Natural Alternative for Sugar Replacer. *The Third International Congress on Interdisciplinary Research and Development*, 3.1-3.4
116. Ulbricht, C., Isaac, R., Milkin, T., Poole, E.A. and Rusie, E. (2010). An evidence based systematic review of *Stevia* by the Natural Standard Research Collaboration. *Cardiovascular Haematological Agents in Medicinal Chemistry*, 8: 113-127.
117. USDA, (2015). *Mycotoxin Handbook*, Chapter 6, September 17, 2015, Page 6 – 2, Washington, D.C.

118. Vanek, T., Nepovim, A. and Valicek, P. (2001) .Determination of Stevioside in plant material and fruit teas. *Journal of food composition and analysis*, 14,383-388.
119. Varanuj, Chatsudthipong, Chatchai and Muanprasat (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacology & Therapeutics*, Volume 121, Issue 1, January 2009, Pages 41-54.
120. Vieira, E.R. (1996). *Elementary Food Science*. 4th Ed. New York: Chapman and Hall, 1996.
121. Wild, S., Roglic, G., Green, A., Sicree, R. and King, H (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004 May; 27(5):1047-53.
122. World Health Organization, (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. http://apps.who.int/iris/bitstream/10665/66040/1/WHO_NCD_NCS_99.2.pdf
123. World Health Organization, (2016). Global report on diabetes. http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf
124. Yadav, A.K., Singh, S., Dhyani, D. and Ahuja, P.S (2011). A review on the improvement of *Stevia* [*Stevia rebaudiana* (Bertoni)]. *Canadian Journal of Plant Science*. 91: 127.
125. Yadav, S.K., and Guleria, P. (2012). Steviol Glycosides from *Stevia*: Biosynthesis Pathway Review and their Application in Foods and Medicine. *Critical Reviews in Food Science and Nutrition*, Volume 52, 2012 - Issue 11 Pages 988-998.
126. Yu, H., Yang, G., Sato, M., Yamaguchi, T., Nakano, T. and Xi, Y. (2017). Antioxidant activities of aqueous extract from *Stevia rebaudiana* stem waste to inhibit fish oil oxidation and identification of its phenolic compounds. *Food Chemistry*, 1; 232: 379-386.
127. Yucesan, B., Mohammed, A., Buyukgocmen, R., Altug, C., Kavas, O., Gurel, S. and Gurel, E. (2016). In vitro and ex vitro propagation of *Stevia rebaudiana* Bertoni with high Rebaudioside-A content—A commercial scale application. *Scientia Horticulturae*, Volume 203, 12 May 2016, Pages 20-28.
128. Zahn, S., Forker, A., Krugel, L. and Rohm, H. (2013). Combined use of rebaudioside A and fibres for partial sucrose replacement in muffins. *LWT* -

Food Science and Technology, Volume 50, Issue 2, March 2013, Pages 695-701.

129. Zhang, Q., Yang, H., Li, Y., Liu, H. and Jia, X. (2017). Toxicological evaluation of ethanolic extract from *Stevia rebaudiana* Bertoni leaves: Genotoxicity and subchronic oral toxicity. *Regulatory Toxicology and Pharmacology*, Volume 86, June 2017, Pages 253-259.
130. Zlabur, J.C., Voca, S., Dobricevic, N., Jezek, D., Bosiljkov, T. and Brncic, M. (2013). *Stevia rebaudiana* Bertoni- A Review of Nutritional and Biochemical Properties of Natural Sweetener. *Agriculturae Conspectus Scientificus*, Vol. 78 (2013) No. 1 pp. 25-30.
131. Zulkifli, A.H., Ismail, W.R.W., Bachok, S. and Baba, N. (2016). Low calorie cake: the impact of *Stevia* application in baking product. *An International Multi-Disciplinary Graduate Conference of Terengganu.*

WEBOGRAPHY

- https://timesofindia.indiatimes.com/city/chennai/1-in-10-people-in-Tamil-Nadu-is-diabetic/articleshow/7096511.cms
- http://www.thehindu.com/sci-tech/health/42-lakh-individuals-with-diabetes-in-TN-says-INDIAB-Study/article15591914.ece
- http://www.thehindu.com/news/cities/Coimbatore/coimbatore-district-has-28-lakh-diabetics-says-study/article2032749.ece
- www.biocyclopedia.com

APPENDIX I

DETERMINATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity is determined by measuring the Diameter of Zone of Inhibition (IZD). Paper discs impregnated with specific antibiotics or the test substances were placed on the surface of the Muller Hinton Agar or Rose Bengal chloramphenicol inoculated with the target organisms. The plates were incubated and the zones of inhibition around each disc were measured.

Reagents

a. **Muller Hinton Agar Medium (MHA):**

The medium was prepared by dissolving 33.9 g of Muller Hinton Agar Medium along with 1 gram of agar agar in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121ºC for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured onto 100 mm petriplates (25 ml/plate).

b. **Rose Bengal Chloramphenicol (RBC):**

The medium was prepared by dissolving 32.15 g of Rose Bengal Chloramphenicol medium along with 1 gram of agar agar in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121ºC for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured onto 100 mm petriplates (25 ml/plate).

Procedure

- Fresh culture to be tested was completely swabbed on the surface of the agar plates.
- The provided antibiotic discs was placed on the surface with the help of sterile forceps. Care was taken to ensure that the zone formation to an antibiotic did not interfere with another.
- The plates was incubated at 37° C for 24 hours for antibacterial test and for antifungal test the plates was kept in the room temperature.
- Zone of inhibition was measured with the help of a meter scale and compared with the given standard chart (Bauer *et al.,* 1966)

APPENDIX II

ANALYSIS OF TOXIC SUBSTANCES

Aflatoxins

Principle of LC/MS

Aflatoxin is determined by LC-MS, a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. Even with a very sophisticated MS instrument, HPLC is still useful to remove the interferences from the sample that would impact the ionization. Interface that will eliminate the solvent and generate gas phase ions, then transferred to the optics of the mass spectrometer (FSSAI, 2012).

Test Procedure

Sample Preparation

Weigh 25 g of the sample in to a 250 ml conical flask to this add 5g of AR/GR grade Sodium Chloride and 100mL of aflatoxin extraction. Cap & seal the conical flask with Para film, Shake at about 140rpm on a horizontal shaker for 30 minutes. After shaking keep the flasks standing for 5minutes for settling of suspended particles if any. Filter all the extraction solution through a fluted filter into a 150ml Beaker. From the beaker take 15ml of the filtrate into 50ml graduated measuring cylinder and to this add 30ml of water. Add 40ml of De-ionized water to the cylinder and mix.

Immuno-Affinity Column Clean-up

Attach an aflatest-column to the pump stand. Pipette 10ml of filtrate on the column and allow it to absorb on column. Once the entire filtrate has passed through the column, rinse the column with 10 ml of de-ionized water, repeat de-ionized water rinse. Place a 2 or 4 ml vial under the tip of the column and add 1mL of methanol to the column collect the elute and again add 1ml water to the column and collect the elute in the same 2 or 4 ml vial. This sample is now ready for injection into the HPLC-MSMS.

Injection Sequence

- Inject calibration standard(s)
- Inject the recovery sample
- Inject the blank sample and verify the absence of analytes above 5% of the recovery or sample concentration(s).
- Inject sample extract(s).
- Re-inject the calibration standard at the appropriate level at least after every 20 injections and at the end of the run to verify instrument response (FSSAI, 2012).

Ochratoxin

Principle

Test portion is extracted by blending with acetonitrile–water. The extract is cleaned up by passing through an immunoaffinity column. Ochratoxin A (OTA) is eluted with methanol, further purified and identified by LC, and quantified by fluorescence.

Extraction

Weigh, to nearest 0.1 g, ca 25 g test portion of sample into blender jar B(c). Add 100 mL extraction solvent C(c). Cover and seal blender; blend for 3 min. Filter extract through filter paper B(h).

Immuno affinity Column Cleanup

Pipet 4 mL filtrate into 100 mL glass beaker (or similar) and dilute with 44 mL PBS C(m). Connect immuno affinity column B(l) to vacuum manifold B(e), and attach reservoir B(f) to immuno affinity column. Add diluted extract to reservoir and pass through immuno affinity column at ≤5 mL/min flow rate. The immune affinity column must not be allowed to run dry. Wash beaker and column with 10 mL water, remove from vacuum manifold, and place over silanized vial B(a).

Elute OTA into silanized vial with four 1 mL portions methanol C(e). Evaporate elute to dryness over steam bath, under N. Redissolve in 1mL injection solventC(f), which has been filtered through 0.2 µm filter. Transfer to LC vial.

LC determination

- Calibration graph.—Prepare calibration graph at beginning of each day test portions are analyzed.
- Preparation of standards.—Pipet 200 µL10 µg/mL OTA calibrant solution C(a) into glass vial and dilute to 1mL with 800 µL toluene–acetic acid C(q) to give 2 µg/mL OTA solution. Pipet 100 µL2 µg/mL OTA solution into silanized glass vial B(a). Evaporate solvent under stream of N. Redissolve in 10 mL injection solvent C(f) that has been filtered through 0.2 µm filter. This gives 20 ng/mL OTA solution.
- From this solution, prepare 5 LC calibrants in separate 5 mL volumetric flasks. Dilute each calibrant (Cal) to volume (5 mL) with filtered injection solvent C(f).
- Operating conditions.—When column B(l) and mobile phase specified C(o) were used, the following settings were appropriate: Flow rate, 1 mL/min; column oven temperature, 45 ± 1°C; fluorescence detection, 460 nm emission wavelength, 333 nm excitation wavelength; injection volume, 100 µL.

Evaluation

Determine, from calibration graph, masses in ng of OTA in aliquot of test solution injected onto the LC column, MA (AOAC, 2000)

APPENDIX III

STANDARD PROCEDURE

RAVA KHEER

Ingredients

Milk-100 ml

Water- 250 ml

Rava- 20 g

Almonds- 2 g

Raisins- 2 g

Cashew nuts- 2 g

Stevia rebaudiana leaf infusion- 7.5 ml

Ghee- 10 g

Method

- Roasted cashew nuts, raisins and almonds in ghee.
- Roasted rava in ghee for 3-4 mins till it becomes fragrant.
- Boiled the water and added the rava on medium flame stirring continuously to avoid the lump formation and cooked.
- Added milk and cooked for 2 mins till the semi-solid consistency was reached.
- Added the *Stevia rebaudiana* leaf infusion and mixed.
- Garnished with cashew nuts, raisins and almonds and mixed.
- Switched off the flame and served.

MOONG DHAL PAYASAM

Ingredients

Moong dhal- 20 g

Milk- 100 ml

Water-250 ml

Stevia rebaudiana leaf infusion- 7.5 ml

Almonds- 2 g

Raisins- 2 g

Cashew nuts- 2 g

Ghee- 10 g

METHOD

- Roasted cashew nuts, raisins and almonds in ghee.
- Roasted moong dhal for 3-4 mins in a thick bottomed pan.
- Boiled moong dhal in water till soft.
- Mixed with boiled milk and simmered for 2 mins.
- Added the *Stevia rebaudiana* leaf infusion and mixed.
- Garnished with cashew nuts, raisins and almonds.
- Switched off the flame and served hot.

APPENDIX IV

SCORE CARD FOR _____

SENSORY EVALUATION

Name: **Date:**

Age:

Class:

CRITERIA	SCORES				9- Point Hedonic Scale	
	Standard	Variation 1	Variation 2	Variation 3	9	Like Extremely
Appearance					8	Like Very Much
Colour					7	Like Moderately
Flavour					6	Like Slightly
Consistency					5	Neither Like nor Dislike
Taste					4	Dislike Slightly
Overall Acceptability					3	Dislike Moderately
					2	Dislike Very Much
					1	Dislike Extremely

APPENDIX V

ETHICAL CLEARANCE

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam
Institute for Home Science and Higher Education for Women
University
(Estd. u/s 3 of UGC Act 1956)

Chairman
Dr. S. Ramalingam
Principal, PSG Institute
of Medical Sciences
& Research, Coimbatore

Member Secretary
Dr.S.Uma Mageshwari
Associate Professor,
Department of Food Service
Management & Dietetics

Members
Dr. S. Kowsalya
Dr.P.R.Padma
Mr. K.Arulmoli (Legal Expert)
Dr. N.S. Rohini
Dr.A. Saraswathy
Mrs. V. Mangayarkarasi
Dr.Subhashini K. Sripathi
Mrs. S. Radha Devi
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.AnithaSubash

19th March 2018

To
Ms. Subhasree. B
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Subhasree,

Ref: Your proposal No. IHEC/17-18/FSN/17 "Evaluation and Development of Beverages and Desserts substituted with *Stevia rebaudiana* leaves" submitted for approval of the IHEC on 14th December.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/17-18/FSN/17 "Evaluation and Development of Beverages and Desserts substituted with *Stevia rebaudiana* leaves" submitted by you. The Approval number for the same is AUW/ IHEC/ FSN -17-18/XPD/17.

We wish you all the best in your research endeavours.

Regards.

Uma Mageshwari
Dr.S.Uma Mageshwari
Member Secretary

Institutional Human Ethics Committee