DEVELOPMENT OF HERBOMIX AND EFFECT OF SUPPLEMENTATION ON LIPID PROFILE OF SELECTED SUBJECTS

BY

MISS. KHADKE PUNAM ARUNRAO
B.Sc. (Home Science)

DISSERTATION

Submitted To

The Marathwada Agricultural University, Parbhani

In partial fulfillment of the Requirement

For the Degree of

MASTER OF SCIENCE

IN

HOME SCIENCE
(FOODS AND NUTRITION)

DEPARTMENT OF FOODS AND NUTRITION
COLLEGE OF HOME SCIENCE
MARATHWADA AGRICULTURAL UNIVERSITY
PARBHANI-431 402 (M.S.) INDIA
2010

Dedicated to my beloved late sister, Nilu whose eternal memories will always reside deep down inside my heart

CANDIDATE'S DECLARATION

I, hereby declare that
this dissertation or part of
there of has not been
submitted by me to other
University or Institute for
a degree or diploma.

Place : Parbhani

Date :20 / 05 /2010

Khadke

(Khadke Punam Arunrao)

CERTIFICATE-I

This is to certify that **Miss. Khadke Punam Arunrao** has satisfactorily prosecuted her course of research for a period of not less than two semesters and her dissertation entitled "**Development of Herbomix and Effect of Supplementation on Lipid Profile of Selected subjects**". Submitted by her is the result of original research work and is of sufficiently high standard to warrant its presentation to examination.

I also certify that, the dissertation or part of there of has not been previously submitted by her for the award of degree of any University.

Place: Parbhani

Date: 20/05/2010

Dr.(Mrs.) Khan T. N.
Research Guide
Associate professor
Department of foods And Nutrition
Collage of Home science
M.A.U. Parbhani.

CERTIFICATE-II

This is to certify that the dissert5ation entitled **"Development of Herbomix And Effect Of Supplementation On Lipid Profile OF Selected Subjects"** submitted by **Miss. Khadke Punam Arunrao** to Marathwada Agricultural University, Parbhani in partial fulfillment of the requirement for the degree of **Master of Science** (Home Science) in the subject of **'Foods and Nutrition'** has been approved by the Student Advisory Committee after oral examination in collaboration with the external examiner.

(Sarambekar H.L)
(External Member)

Dr.(Mrs.) Khan T.N.
Research Guide
Associate professor
Department of foods And Nutrition
College Of Home Science
M.A.U. Parbhani.

Members of Advisory Committee

Head
Department of Foods and Nutrition
College of Home Science
M.A.U. Parbhani

Dr.(Mrs.)Rohini Devi
Professor & Head
Department of Foods and Nutrition
College Of Home Science
M.A.U. Parbhani.

Dr. (Mrs.) Nalwade Vijaya M.
Associate professor
Department of Foods and Nutrition
College Of Home Science
M.A.U. Parbhani.

Prof. D. Murali
Associate Dean And Principal
College of Home Science
M.A.U. Parbhani

Dr.(Mrs.) Asha Arya
Associate Professor
Department of Foods and Nutrition
College Of Home Science
M.A.U. Parbhani

ACKNOWLEDGEMENT

Emotions cannot be adequately expressed by words, only acknowledgement are much more than what I am expressing here.

With heartfelt gratitude, I acknowledge the invaluable guidance bestowed on me by guide Dr. (Mrs.) T.N. Khan, Associate Professor, Department of Foods and Nutrition, College of Home Science, Marathwada Agricultural University, Parbhani. Her paternal affection and vigilant care have always encouraged me to persue right path in my work. Her profound knowledge of the subject, dedication to work and amazing foresight will always inspire in my future endevours.

My sincere thans to Prof. D. Murli, Associate Dean and Principal, College of Home Science, Marathwada Agricultural University, Parbhani for providing facilities in the college of carry out the research work.

I wish to evince my grateful thanks to Dr. (Mrs.) Rohini Devi, Professor and Head of the Department of Foods and Nutrition for providing the facilities in the department to carry out the research.

I also appraise my deep gratitude to Dr. (Mrs.) V.M. Nalawade, Associate Professor of Foods and Nutrition, College of Home Science, Parbhani for their valuable suggestions, guidance and co-operation during the period of research work.

My special and heartful thanks to all the subjects who participated in the present study for their co-operation without this, work could not have been completed. I would like to record my appreciation and gratitude to all the panel members for their unstinted co-operation in evaluating the acceptability of herbomix.

I express my special thanks to Dr. (Mrs) Farzana Assistant Professor, Mrs. B.J. Ansari, Supervisor, Mrs. J.P. Nerlekar and Mrs Varsha Zanwar, Research Associate, Department of Foods and Nutrition, College of Home Science, Parbhani

I am very much thankful to Mr. S. K. Chopade for his meticulous typing of thesis with utmost care.

I owe a deep sense of gratitude to my beloved parents and brother Dr. Amit Khadke for their kind co-operation help, un-resting encouragement unfailing love, moral support, unflenching affection in building up my educational career and standing always with me in each and every improbable moment in my life.

I am also thankful to my friends and well wishers for their kind co-operation.

Place : Parbhani

Date : 20th May, 2010

(Khadke Punam Arunrao)

LIST OF TABLES

LIST OF FIGURES

CONTENTS

INTRODUCTION

CHAPTER I

INTRODUCTION

"A healthy body is the quest chamber for soul, a sick body is a prison" (Bacon)

Traditional medicine has been described as one of the surest means to achieve total health care coverage for the world's population. As it is safe, economical and feasible method. The world health organization has defined traditional medicine as "A system comprising therapeutic practices that have been in exercise often for hundreds of years before the development and spread of modern medicine and still in use today" (WHO, 1991). India has one of the worlds most sophisticated indigenous medical culture. The medical heritage is many centuries old, millions of Indians depend on it even today for treating various health problems.

Plants have been used by mankind as a source of drugs to combat diseases for several thousand years in the classical system of medicine such as Ayurveda, Siddha, Unini, homoeopathy. Ayurveda is a comprehensive natural health care system that is originated in India more than 5000 years ago. Ayur means 'life' and veda means 'knowledge'. The results of ayurveda treatment are encouraging effective for various ailments, chronic disorders associated with aging process. The treatment is simple, economic and also do not have prevalence of toxic side effects.

The impact of industrialization, the rise of science and technology , loss of patience, fast life, going for quick remedies and without bothering for their side effects has totally curtailed our involvements with nature but recently there has been shift in universal trend from synthetic to herbal medicine which can be said as "return to nature". It is becoming increasing clear that there is a strong relationship between the food we eat and our health. Plant contain a large number of phytonutrients that have the potential

for offering protection against a range of non-communicative diseases like diabetes, cancer, cardiovascular diseases and cataract. It also provides antirudants which prevent different diseases.

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as defense system against disease. Phytochemicals are divided in to two groups which are primary and secondary constituents, according to their functions in plant metabolism primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, phenolic compounds, lavonoids, tannins, flavonoids, glycosides and saponins (Krishnaiah *et al.*, 2007).

Epidemialogical studies have linked with low incidence of various non-communicable diseases (NCDs) the inverse association between high intake of fruits and vegetables and lower incidence of NCDs may be attributed to number of foods factors such as folate antioxidants, vitamins or other constituents namely fiber, potassium, flavonoids and other phytochemicals. Although the biologically active phytochemicals have been reported to present in a wide variety of plant foods. These phytochemicals are reported to prevent disease mainly through their functions as antioxidants, detoxifiers, neuropharmocological, immuno-potentiating agents. A large number of antioxidants occur in foods. Besides nutrients such as β-carotene, vitamin-C and Vit. E a number of carotenoids, flavonoids and phenols also occur naturally in foods and act as antioxidants, Vitamin c is considered important in preventing cellular damage from oxidatae stress.

Herbs are most often defined as any part of a plant that is used in the diet for its aromatic and medicinal properties. Most exciting development in the field of herbals, use in allopathy and in household remedies is forcibly changing the understanding of the world 'Herb' in the form of food shelter,

removal of CO_2 waste, cooling the atmosphere also cure from ailments and bring people more nearer to the mother nature and play an important role to keep people healthy.

Herbs have been prized for their pain relieving healing abilities, and curative properties. In India different herbal plants are used for medicinal purposes are Ashwaganda (*Withania somnifera*), Bottle gourd (*Lagneria siceraria*), Curry leaves (*Murraya koenigii*), Amla (*Emblica officinalis*), Garlic (*Allium sativum*), Safflower(*Carthamus tinctorius* L.), Noni(*Morinda citrifolia),*Bale*(Agel marmelose),* Tulsi *(ocimum sanctum).* Mint (*Mentha arvensis),* Ginger *(Zingiber officinale),*etc.

Amla (*Emblica officinalis*) is native of India, Malaya and China. It belongs to Euphoribaceae family. Amla is rich source of ascorbic acid containing about 300-1000 mg/100 g (Manny and Shadaksharaswamy, 1997) along with total phenolics (Karl, 1998).The fruit is either consumed as a fresh or in the preserve, form like candy, dried chips, jelly, pickle, squash and syrup (Karla, 1988). Amla is also used for various ayurvedic products like trifla and Chavanprasha (Premi *et al.*, 1999). Amla contains innumerable constituents in varying amounts falling in broad classes of alkaloids, benzoids, derivatives, diterpens and interpens, furanolactones, flavonoids and sterols. Amla possesses antioxidative activity this may be because of presence of high concentration of flavonoids, tannoids and vitamin-C which show effective results against oxidative damage (Haque *et al.*, 2001).

Amla fruit is acrid, cooling, refrigerant, diuretic and laxative. The dried fruit is useful in hemorrhage, diarrhea and dysentery. It is used as laxative to relive constipation in piles in treatment of leukorrhea and atheroscterosis. It has been use in many local traditional systems, such as Chinese herbal medicine, Tibetan medicine and Ayurvedic medicine (Potwale *et al.*, 2008).Amla has beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anaemia and various other diseases. Similarly,

it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antiussive and gastroprotertive. Additionally, it is useful in memory enhancing, ophthalmic, disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and is an antimicrobial (Khan, 2009).

Bottle gourd (*Lagenaria siceraria*) is a common vegetable in India belonging to family cucurbitaceae, rapid growing climbing annual herb grown throughout India and fruits are available in the market throughout the year. It is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health (Rahaman, 2003).

The edible portion of fruit is good source of vitamin-B complex and contain highest source of choline level – a lipotropic factor, a healer of mental disorder. Phytochemical screening revealed the presence of flavonoids, sterols, cucurbitacin saponin, polyphenolics, proteins and carbohydrates. Bottle gourd contains variety of essential phyto-nutrients and rich in minerals, trace elements, dietary soluble fiber pectin, traditionally fruits are used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poison and scorpion strings, alternative purgative and helps in constipation.

Safflower (*Carthamus tinctorius L.*) is a flowering plant belonging to the family Asteraceae of broad group compositae. The colour of flowers varies from whitish yellow to red orange and the most common is yellow. Safflower florets contain two pigments carthamin which is red and insoluble in water, safflower yellow carthamidin which is soluble in water. The yellow (20%) and red(2%) pigments extracted from safflower are widely used as stain, additive in food, beverage, cosmetics, printing and dyeing (Wang and Fan, 1989)

The use of safflower was recorded in china approximately 2200 years ago both as food and medicine. The powder prepared from safflower petals was used as an ingredient in some traditional Indian food items like flavored milk , herbal tea, kesari coconut burfi, basundi, limerice, khichadi, cabbage and potato curry (Sarojini *et al.,* 1995). petals regarded as a stimulant for blood circulation , healing fractures, strains for various female maladies (Kaffka *et al.,* 2000)

Tulsi (*Ocimum sanetum*) belongs to labiateae family. It has been used in Ayurvedic systems for thousands of years . Medicinal, religious and culinary use of tulsi has been documented for centuries in Asia, China the middle east, North Africa and Australia. Phytochemical compounds in tulsi leaves are eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (phenylpropanoid) . (Maime, 2004).

The leaves contains ascorbic acid (83 mg/100 g), urosolic acid, apigerun, luctolin, apigenin along with terpene neurobosolic acid which has anticancer properties. It is useful in cardiopathy, haemopathy, leuoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, vomiting, lumbago, hiccough opthalmia, gastropathy in children, genilo urinal disoders all skin disorders (Dehspande,2008). Tulsi has a positive effect over blood pressure and also a detoxicant, its regular use prevents heart attack (Agarwal, 1996).

Honey has been recognized as one of the most natural home remedies since ancient times, to treat a wide range of ailments. It has been demonstrated that honey serves as a source of natural antioxidants, which are preventing deteriorative oxidation reactions in foods. The antioxidants in honey prevent cholesterol from being moved out of the blood and into the lining of the blood vessels. Honey contains natural minerals and vitamins which help to metabolize undesirable cholesterol and fatty acid on the organs and tissues into the system, hence preventing obesity and promoting better health. The vitamins present in honey are B_6, thiamin, niacin, riboflavin,

panthothenic acid and certain amino acids. The minerals found in honey include manganese, phosphorus, potassium, sodium and zinc.

Herbs represent the most effective Ayurvedic approach to healing illness. Their action is strongest when they are fresh, also it can be used in the form of decoction, influsions, tea, powder and pills.

Thus efforts were made to develop herbomix by utilizing herbs like amla, bottle gourd, safflower petals, tulsi and to assess the effect of supplementation of herbomix on serum lipid profile of selected subjects, by keeping in view the easy availability, safety and economical value of herbs.

The objectives of the study are as follows

1 To develop and evaluate the acceptability of herbomix .

2 To determine the nutritional composition of highly accepted herbomix .

3. To determine the nutritional intake and lipid profile of selected subjects .

4. To assess the effect of herbomix supplementation on lipid profile of selected subjects.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Medicinal plants are local heritage with global heritage. World is endowed with rich wealth of medicinal plants. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities. In last few years there has been an exponential growth in field of herbal medicine and these drugs are gaining popularity in both developing and developed countries because of their natural origin and less side effects.

The available literature related to the present study is described here under the following heads.

1. Studies on utilization and incorporation of selected herbs in various products.
2. Studies on nutritive value and antioxidant capacity of selected herbs.
3. Studies on therapeutic value of selected herbs.

2.1 Studies on utilization and incorporation of selected herbs in various products

Ayurveda, traditional Indian medicinal system has a long history and is one of the great living traditions. Many medicinal plants in Ayurvedic medicines are believed to be used in strengthening the human immune system and formulation based on such plants play an important role in modern health care, particularly where effective and safe treatment is not available. Along with this medicinal properties herbs are used for many other purposes including beverages such as tea, dyeing, repellents, fragrances, cosmetics, smoking and industrial uses. The demand for plant based medicines health products, pharmaceuticals, food supplements, cosmetics are increasing due to growing recognition that the natural products are non toxic have less side effects and easily available at affordable prices.

Herbs are used in diet for its aromatic properties, also herbs are of great use in cookery as flavoring agent and preservatives.

2.1.1 Studies on utilization and incorporation of amla (*Emblica officinalis*) in various products.

Instant aonla pickle without oil was prepared by utilizing two varieties of aonla (Desi and Chakaiya) the prepared pickle was stored in glass jars for 15-20 days at 30^0C temperature. Results of study indicated that retention of ascorbic acid was better in chakaiya variety (69.22 mg/100 g) than the desi (Premi *et al,*2002). Bajaj and Satwadhar (2006) prepared the herbal drink from amla juice, tulsi juice, ginger juice, mint juice and cumin seed extract. It was found that prepared herbal drink was nutritionally rich, economical, consumer acceptable and had lots of therapeutic value.

Sawant *et al.*. (2006) prepared chayawanprash with addition of amla, dry dates, ashwgandha and shatawari. The sensory attribute indicates best consistency was obtained and product found to be acceptable.

Tiwari *et al.* (2006) reported that sulphide treated amla (*Emblica officinalis* Geartn) fruit pulp with or without in bottle pasteurization was used to prepare three products namely ready to serve beverage (RTS), squash and jam at the interval of three months during ambient storage for nine months because of its excellent therapeutic and nutritive value.

Extraction of amla juice by different method was studied by Vijayanand *et al.* (2006). The results indicated that amla juice stored for six months at room temperature was self stable thus it can be used for preparation of beverages, chayanprash and as an acidulant in traditional food preparation.

2.1.2 Studies on utilization and incorporation of bottle gourd (*Lagenaria siceraria*) in various products.

Garg *et al.* (2008) developed two fruit drinks rich in vitamin C. The first drinks was prepared using desi aonla, apple and ginger were used in the

80:15:05 proportion while second fruit drink was prepared using banarasi aonla, apple and ginger was used in 70:25:05 proportion. The results of the study indicated that the product was acceptable for six months when stored at ambient conditions of 28 ± 5 C and 62± 5% in 200 ml glass bottles.

Deore *et al.* (2008) studied the suitability of bottle gourd for juice and powder. The processed bottle gourd was stored for 90 days and determined the chemical changes during storage period. . Results of study revealed that the juice pasteurized at 800^0C, mixed with 100 ppm sodium benzoate and stored at cold (05 ± 20^0C) temperature, was found acceptable upto 90 days of storage. The bottle gourd powder was packed in 200 guage polythene bags and stored at ambient (30 ± 20^0 C) temperature was found acceptable up to 90 days of storage.

Sawate *et al* (2008) prepared ready to serve (RTS) beverage and juice from bottle gourd and evaluated their qualities at different storage conditions. It was found that the treatment of blanching is effective for the extraction of juice to retain the natural and fresh colour of the juice and also found organoleptically acceptable. A good quality RTS beverage can be prepared by using 15 percent level of bottle gourd juice having 0.32 percent acidity. The developed RTS beverage can be stored at refrigerated condition for 2 month and at ambient condition for one month without affecting its quality attributes. Processing technology used for bottle gourd RTS beverage preparation is beneficial to the consumer with regard to nutritional and therapeutic value.

2.1.3 Studies on utilization and incorporation of safflower petals (*Carthamus finetorius*) in various products.

In Afghanistan and India safflower was used mainly as an edible oil. The florets were added to rice, bread and pickles to give them an attractive orange colour (Solunkhe *et al*1992).

Wu *et al.* (1993) observed that safflower yellow is very safe for cosmetics. It can be used in Cakes, beverages, cosmetics and medicinal tablets. They also indicated that safflower red used to colour medicines and foods.

Revanwar (1996) carried out a study on the use of safflower yellow pigment in Shrikhand at 1.0, 2.0, 3.0 and 4.0 per cent level. The 2.0 per cent level of addition of safflower pigment was considered to be more accepted by the panel members than the other levels.

Wang and Du Lijie (2001) reported that safflower would be used as dye in the candy, cake, dairy products, spices, wine, paste and flour food. They also indicated that safflower used as dye in hairoil, milk, perfumes, bath soap and dye the furniture.

Khan (2005) conducted a study on incorporation of safflower petals in decoction and food products. The safflower petals powder was incorporated in decoction 1, 1.5 and 2 per cent concentration. From the study it was concluded that incorporation upto 2 per cent concentration of safflower petals is accepted. The recipies selected for incorporation of safflower petals powder were *chapati, dhapata, shira, upma, coconut chutney, seasum chutney* and *chiwda*. The varying levels of incorporation of safflower petals powder in selected levels were 5, 10, 15 and 20 per cent. From the study it was concluded that the incorporation of safflower petals powder in food products are accepted.

Ingole (2007) studied the Chutney prepared by incorporation of different levels of safflower petals powder. Chutneys selected for incorporation of safflower petals were *seasum chutney, coconut chutney, groundnut chutney, niger seed chutney* and *linseed chutney*. Safflower petals powder was incorporated in chutney at varying levels i.e. 5, 10, 15, 20 and 25 per cent.

2.1.4 Studies on utilization and incorporation of tulsi (Ocimum Sanctum) in various products.

Uhl (1996) reported that tulsi is widely used as the most important flavouring ingredient in South-East Asian Cuisine especially in Thai curry paste and spicy soups because of its spicy and lemony notes.

Pruthi (1998) reported that tulsi is used in certain cheeses, fish, soups, tomato cocktail, cooked cucumber dishes and squash. Chopped basil is sprinkled over lamb chops before cooking. The oil of tulsi is employed in all kinds of confectionary, baked products and condemenatory products like chilli sauce, tomato paste, pickles, and sausages. It is often used in pizza topping.

Juantachote and Berghofer (2005) reported that the holy basil exhibited a protective effect as natural food antioxidant and food additive and increased the shelf-life or stabilization of foods by preventing lipid oxidation in cooked ground pork.

Anbarasu and Vijayalakshmi (2007) conducted a study to improve the shelf life of protein rich tofu using extracts of tulsi commonly available in rural areas to benefit Indian rural population. Aqueous extract of tulsi was added during the preparation and storage of force to prolong its shelf life. The results indicated that the shelf life was successfully extended for 7 to 8 days from 3 to 4 days without refrigeration.

Vani *et al.* (2009) reported that the tulsi is cultivated for its remarkable essential oil which is used in medicinal application, aroma therapy treatment, perfume industry and as food seasoning and flavouring.

From the above studies it can be said that herbs are mainly used as flavouring and aromatic as they are rich source of essential oil. Herbs acts as preservative, natural colorants and antioxidants in food product. Besides role in cookery herbs are used in cosmetics, perfumes, soaps, fungicides and in medicines.

2.2 Studies on nutritive value of selected herbs

The fruits and vegetable contain number of food factors such as folate, antioxidants, vitamins and other constituents namely fiber, potassium, flavonoids and other phyto-chemicals. These phyto-chemicals are reported to prevent disease mainly through their functions as antioxidants, detoxifiers, neuro-pharmocological, immuno potentiating agents. A large number of antioxidants occur in foods besides the nutrients such as βcarotene, vitamin C and vitamin E.

2.2.1. Studies on nutritive value of amla (*Emblica officinalis*)

Bhattacharya *et al.* (1999) observed that the amla fruit contains different bioactive components mainly quercetine, phyllambic compound, gallic acid, tannins, flavonoids, pectin and vitamin C.

Jain and Khurdiya (2004) found that the *Emblica officinalis* contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest amount of vitamin C (478.56 mg/100 ml).

Vanitha *et al.* (2005) evaluated antioxidants capacity of some plant extracts for their application in biscuits. The plant foods viz. amla (*Emblica officinalis*), drumstick leaves (*Moringa oleifera*) and raisins (*Vitis vinifera*) were used as source of natural antioxidants. All the three extracts exhibited a high percentage of antioxidant activity evaluated using β-carotene linoleic acid in vitro system, compared to synthetic anti-oxidants. Addition of plant extracts from the three plant foods gave excellent antioxidant effect on the biscuits.

Asadul Haque *et al* (2006) studied the total antioxidant capacities of some local common vegetables, spices and tea. Total antioxidant capacity tested by Randox kit showed higher value for black tea (*Thea sineosis*) amla (*Phyllanthus emblica*) and red chilli (*Capasicum annum*) having high values of 707.4, 306.6 and 235.8 mmol Trolox/ kg of dry matter respectively and among vegetables relatively high values in unblanched amaranthus

(*Amaranthus paniculata*) leaf, drumstick (*Morringa pterigossperma*) leaf and mint (*Mentha viridis*) leaf having values of 31.2, 29.7 and 18.5 mmol of Trolox/kg of dry matter, respectively.

Xiaoli Liu *et al.* (2007) evaluated the phenolic contents of methanolic extracts of amla fruit from six regions in china. The antioxidant activities of these extracts were also evaluated. It was found that the methanolic extracts of emblica fruit from some selected regions exhibited stronger antioxidant activities compared to those of the commercial compounds (quercetin BHA). Hence, it can be concluded that it might be considered as a potential plant source of antioxidants.

Potawale (2008) reported that the pulp obtained from whole amla fruit was 90.67 per cent which contains moisture (70.5%), acidity (3.28%), total sugars (5.09%), reducing sugars (5.08%), tannins (2.73%), pectin (0.59%), protein (0.75%), mineral (2.922%), phosphorus (0.027%), potassium (0.368%), calcium (0.059%), magnesium (0.248%) and iron (0.0044%) where as amla juice contains total soluble solids 23.8 per cent.

Khan (2009) reported average percentage composition of the fruit pulp of *Emblica officinalis* as moisture (8.12%), protein (0.5%), fat (0.1%), mineral matter (0.7%), fibre (3.4%) carbohydrate (14.1%), calcium (0.05%), phosphorus ((0.02%), Iron (12 mg/100g) nicotinic acid (0.2 mg100g) and vitamin C (600 mg/100 g).

2.2.2. Studies on nutritive value and antioxidant capacity of Bottle gourd (*Lagenaria siceraria*)

Chang *et al* (1995) reported that bottle gourd contain more proportion of soluble dietary fibers (SDF) than insoluble fibers. SDF are having profound effect in lowering serum cholesterol.

Shriwaikar *et al* (1996) studied the chemical constituents of bottle gourd. Two sterol, fucosterol and compesterol were identified, isolated from petroleum ether extract of dried fruit pulp of bottle gourd.

Rumeza *et al* (2006) studied the importance of vegetable in human nutrition. The proximate compositions, minerals and vitamins of different vegetables were studied. The results of the study revealed that the bottle gourd contains moisture (94.5 ± 0.06), protein (1.2 ± 0.06), fats (0.2 ± 0.01), carbohydrates (3.75 ± 0.03), fiber (0.7 ± 0.01), ash (0.5 ± 0.01) g/100 gm and energy 15 ± 0.0 kcal. The amount of Ca (12 ± 0.03),P(37 ± 0.01), Na (1.7 ± 0.01), K (87 ± 0.02), Cr (0.05 ± 0.06) and Fe(0.8 ± 0.01) mg 100 g were recorded. The values recorded for thiamin, riboflavin, niacin and ascorbic acid were 0.03 ± 0.01, 0.05 ± 0.08, 0.3 ± 0.01 12 ± 0.07 (mg/100 g) respectively.

Deshpande *et al* (2007) studied the free radical scavenging activity of bottle gourd. The results of the study indicated that the fresh juice of fruit showed radical scavenging activity. Whereas 100 and 1000 times diluted juice did not show any radical scavenging activity.

Ojiako and Igwe (2007) evaluated proximate and elemental compositions using flavours produced from seeds of cleome rutidosperma (DC), *Lagenaria siceraria* (mol) and cucurbia maxima (Duch) revealed the presence of alkaloids steroids, pentose and reducing sugars in all seed types. Results also showed that the seeds of *C. rutidosperma, L. siceraria and C. maxima* contained carbohydrates (74.43% 45.93% and 24.30%), fats (7.20%, 38.92% and 51.49%) respectively. The major contents of the defatted seed flour found to include potassium (39.00 ppm, 19.50 ppm, 39.00 ppm), sodium (23.00 ppm, 11.50 ppm and 11.50 ppm) and calcium (18.00 ppm, 12.00 ppm and 15.00 ppm) respectively. They were also found to contain zinc and iron.

Ersato and Mbwambo (2009) studied the antioxidant activity of *Lagenaria siceraria* fruits. In this study the antioxidant effect of fresh and dried fruits of *L. siceraria* was evaluated using ethyl acetate and n-butanol extracts of fresh and dried fruits. Results indicated that ethyl acetate (EA)

extract of fresh fruits exhibited higher radical scavenging activity than other samples. At 0.1 mg/ ml the order of activity was EA dried fruits (70%), Bt dried fruits (71.8%) ≤ Bt fresh fruits (72%) < EA fresh fruits (81.6%) < Gallic acid (88.5%). Results indicate slight difference in activity it can be said that taking fresh or dried fruits of *L. Sileraria* may relatively give similar antioxidant effects.

2.2.3 Nutritive value and Antioxidant capacity of Safflower petals (*Carthamus tinetorus*)

Wang and Fan(1989) reported that safflower pollen is rich in more than 30 kinds of macro and micro elements which are deficient in the body of old people and children such as iron, calcium, magnesium, manganese, zinc, molybedenum, selenium, silicon and copper.

Nagraj (1993) examined three Indian safflower cultivators for their seed and oil quality characters. The oil content ranged from 26.32% the protein content from 21.23% and linoleic acid ranged from 74.78% of the fatty acid composition.

Shrinivas *et al* (1999) had undertaken a study to examine the safflower petals of Indian origin for carthamin oil, protein and fatty acids. Results of the study revealed that petals oil contained was 4.0 – 5.0 per cent. The fatty acid composition revealed the presence of a linolenic acid 15.19 per cent, palmitic acid 14 – 16 per cent. Gamma linolenic acids 2.3 per cent and decodcanic acids 2.5 per cent. were present.

Gopalan *et al* (2000) reported that the safflower leaves contained moisture 91.1 gm, protein 2.5 gm, fat 0.6 gm, mineral 1.3 gm, carbohydrate 4.5 gm, energy 33 kcal calcium 185 gm and iron 5.7 gm/100 gm.

Matsuba *et al* (2003) investigated the effect of the antioxidative activity of a methanol extract of safflower in rats using a low vitamin E diet. It was confirmed that the liver and serum malondialdehyde (MDA) values were decreased due to daily oral administration of fractions from the

methanol extract of safflower petals. Results confirm that safflower petals contain a component that reduces peroxylipid and lipid metabolism.

2.2.4 Studies on nutritive value and antioxidant capacity of Tulsi (*Ocimum sanctum*).

Ntezurubanza *et al.* (1985) indicated the presence of five fatty acids viz. stearic, palmitic, oleic, linoleic and linolenic acids in the oil of tulsi.

Dhananjay *et al.* (1996) analysed trace elements of tulsi (*Ocimum sanetum*). gulvel (*Tinospora cardifolia*), bitter neem (*Azadeiracha indica*), Kanher (*Nerium andicum*), Vekhand (*Acorus calamus*) and Peacock's feather (Ash). It was found that zinc, manganese and sodium were significantly higher in tulsi leaves as compared to that of other plants.

Hussain *et al* (2001) analysed the antioxidant property of aqueous extract of tulsi in diabetic rats. It was found that there was decrease in lipid peroxidation products (LPO) formation and increase in antioxidant enzymes Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) Glutathione Transferase (GT) and one antioxidant reduced glutathione (GSH) in plasma and rat liver, lung, kidney and brain. Hence it was concluded that the decrease in LPO and increase in GSH, SOD CAT, GPX and GT clearly shows the antioxidant property of tulsi.

Gupta *et al* (2002) reported that tulsi in India is a local herb containing potent antioxidants, flavonoids (Orientin, vicenin) and phenolic compounds (eugenol, aplgenin, cirsilineol).

Merai *et al* (2003) determined the nutrient content of 'Sri Tulsi' and 'Krishna Tulsi' leaves powder as moisture 8.80, 6.62 per cent, volatile oil 0.78, 1.24 per cent, protein 21.78, 20.34 per cent, total ash 12.59, 11.44 per cent crude fiber 9.56, 10.28 per cent respectively.

Maime (2004) reported that nutritional components of tulsi includes vitamin A and C, minerals calcium, iron and zinc. Seeds contain fixed oils

having linoleic acid and linolenic acid. Phyto-chemical compounds in tulsi leaves are eugemol, ursolic acid and rosmarinic acid.

Deshpande (2008) reported that essential oil of tulsi mainly contains eugenol (71%), eugemol methyl ether (20%), carvacrol (3%), along with caryophllene, α pinene, nerol, selinene, terpinen 4.01, decyladehyde, β pinene, cineole, linalool, camphor, methyl chavicol and limetrol, and leaves contains ascorbic acid (83 mg/100 gms), urasolic acid, apigenin.

Suanarunsawat *et al* (2009) reported that the essential oil extracted from O*cimum sanctum* leaves predominantly contained phenypropanoid compounds in which engenol and methyl eugenol were the main compounds.

On the whole it can be concluded that fruits and vegetables are excellent gift for human being by nature as having composition of all the essential constituents that are required for normal and good human health. Vegetables and fruits are good source of vitamins like E, C and pro-vitamin A which exhibit antioxidant capacity.

2.3 Studies on therapeutic value of selected herbs

The plant based drugs have been extremely valuable and helpful in the allevation of human sufferings. Some of the claims made by traditional medicinal systems for their drugs have received clinical support from modern medical science (Sukhdev, 1997). As synthetic drugs caused several side effects, now a days the plant based medicines have become popular throughout the world. The contribution of medicinal plants in discovery of new drugs has been enormous in terms of value and activity for treating diseases like cancer, hypertension and several other ailments.

2.3.1 Studies on therapeutic Value of Amla (*Emblica offficinalis*).

Jacob *et al.* (1988) determined the effect of amla supplementation on total serum cholesterol in normal and hypercholesterolemic men aged 35-55 years. The amla was supplemented in diet for a period of 28 days in raw form. Results showed decreased in cholesterol level in both normal and

hypercholesterolemic subjects. Two weeks after withdrawing the supplement the total serum cholesterol levels of hypercholesterolemic subjects raise significantly almost to initial values.

Mathur *et al.* (2002) studied the lipid lowering and antiatherosclerotic effects of *Emblica officianalis* (Amla). Fresh juice of *Emblica officinalis* was administered at a dose of 5 ml/kg body weight per rabbit per day for 60 days. Results of study showed reduction in serum cholesterol, TG, Phospholipid and LDL levels by 82%, 66%, 77% and 90%, respectively. So it can be concluded that *E. officinalis* juice is an effective hypolipidemic agent and can be used as pharmaceutical tool in hyperlipidemic subjects.

Veena *et al.* (2006) administrated the Kalpaamruthaa and Semecaarpus anacardium which is a modified siddha preparation containing *Emblica officinalis*, Semecarpus and cardium and honey to the cancer suffering animals. The results of the study revealed that elevated levels of free cholesterol, total cholesterol, triglycerides, phospholipids and free fatty acids and decreased levels of ester cholesterol in plasma, kidney and liver were reverted back to near normal levels.

Iyer *et al.* (2009) studied the impact of amla supplementation on the glycemic and lipidemic status of Type 2 diabetic subjects. A medium sized fresh amla (approximately 35 gm) was given to selected T_2DM patients on a daily basis for 60 consecutive days. It is observed that amla supplementation led to a significant reduction (37.9%) in the blood sugar values. Favorable response was seen in diabetic subjects after supplementation. There was a 10.4% fall in TC, 14% in LDL-C, 14.4% in non-HDL-C and increase in HDL-C by 5.5% along with this 23.4% reduction was observed in TG. The favourable redistribution of lipoproteins with amla supplementation had a significant positive impact on the atherogenic indices lowering the risk of CHD in diabetic subjects.

Mamila (2009) developed Herbal Composite by utilizing herbs as safflower petals, curry leaves, tulsi leaves, amla and mint. The impact of supplementation of herbal composite on lipid profile and blood pressure was studied. The results of study indicated that supplementation of 20gm of herbal composite for 60 consecutive days decreased significantly total cholesterol, LDL cholesterol and increased HDL, also the systolic and diastolic blood pressure was decreased.

2.3.2 Studies on therapeutic value of bottlegourd (*Legenaria siceraria*)

Chopra *et al* (1992) found that leaves of *Laqenaria siceraria* are taken as emetic in the form of leaf juice or decoction . Leaf juice by adding sugar is useful in jaundice. Crushed leaves are used for baldness and applied on head for head ache, also leaves are used as purgative.

Ghule *et.al* (2006) studied the antihyperlipidemic effect of four different extracts of bottlegourd viz. petroleum ether chloroform, alchoholic and aqueous extracts in triton induced hyperlipidemic rats. Chloroform and alcoholic extracts at two different doses (200 and 400 mg/kg body weight) showed significant effects in lowering total cholesterol, triglyceride and low density lipoproteins along with an increase in HDL level.

Ghule *et.al* (2006) evaluated vacuum dried extract and methanol extract of *L. siceraria* fruit for its diuretic activity. Diuretic activity was assessed by measuring different parameters like total urine volume, urine concentration of sodium, potassium and chloride and found that the both extracts (100-200 mg/kg) showed higher volume and exhibited dose dependent and increased in excretion of electrolytes when compared with respective control.

Hassnpour *et al* (2008) studied the cardio protective activity of *Lagenaria siceraria* and found that fruit power of *L. siceraria* showed good cardio protective effects. L.S. prevents the alterations in endogenous antioxidants (superoxide dismutase, reduced glutathione) and lipid

peroxidation. Where as markers of cardiotoxicity is CK – MB Land LDH were significantly reduced Further the L.S. powder also showed the protection against changes in histopathological alterations.

Mohale *et al.* (2008) studied the antihyperlipidemic activity of isolated constituents from the fruits of *Lagenaria siceraria* in albino rats. The study exhibited that elevated levels of blood cholesterol, triglyceride, LDL were significantly decreased, HDL was significantly increased by the administration of fractions of *L. siceraria* fruit juice in albino rats.

Rane *et al.* (2008) studied the immunomodulatery effects of n-batanol soluble and ethyl acetate soluble fractions of successive methanolic extracts of LSF in rats. Results of study showed that the test fraction possess promising immunomodulatery activity as they increases both primary and secondary actibody titer and also significantly inhibited delayed type hypersensitivity reactions in rats. Both fractions significantly increases total WBC, neutrophils and lymphocytes count. While insignificant changes were observed in monolytes eosinophils and basophils count.

2.3.3 Studies on therapeutic value of safflower (*Carthamus tinctosus*)

Lice (1989) stated that safflower has the effect of envigorating the circulation of blood, mustering the respiratory tract, relieving pain, reducing swelling and stimulating the menstrual flow..

Li Daue *et al.* (1989) indicated that the amount of consumption of dried sunflower in China was 1700 tonnes per year. The flowers were found to be used as dried flowers and extracts. Dried flowers were noticed to be mainly used for preparing drugs to cure diseases of women, coronary heart diseases muscular fatigue etc.

According to traditional Chinese medicine safflower oil has been taken by over 200 patients and the general efficiency is upto 95%. The medicinal oil of safflower has a noticeable curative effect on cardiovascular diseases without side-effects of position. (Yang lizang 1993)

W. Guirong *et al.* (1995) reported that sunflower seed oil contains the greatest amount of linoleic acid had significant medical effectiveness with high blood pressure, coronary heart diseases, arteriosclerosis and obesity of old people. Animal experiments and clinical trials showed that it can decrease the content of blood clot and soften the blood vessels

Sunitha *et al.* (1997) carried out a study to assess the effects of polyunsaturated fatty acid rich vegetable oils like safflower oil and sunflower oil with the unconventional and hypocholesterolaemic rice bran oil on the serum lipid profile of rats. Rats were fed rice bra oil, sunflower oil and safflower oil at 70:30 ratio for a period of 28 days. It showed significantly lower levels of total cholesterol and increased high lipoprotein cholesterol in animal liver. Total cholesterol and triglyceride were also reduced.

According to Li Dajue and Mundel H.S. (1998), safflower dialates arteries, reduces hypertension and increases blood flow and hence oxygenation of tissues. It also inhibits thrombus formation and dissolves thrombi.

Deodhar (2001) evaluated the medicinal value of safflower petals in hypertensive and hyperlipidemic subjects by administering the 0.5 and 1.0 per cent concentration of safflower petals decoction. The results of the study revealed that administration of safflower decoction either at 0.5 or 1.0 per cent level did not exhibit any noticeable effects on anthropometric measurements of subjects. Besides intake of 1.0 per cent concentration of safflower petals decoction reduced significantly systolic and diastolic blood pressure of 30 and 60 days. A significant marked increase in the serum HDL cholesterol was recorded. But other lipids in serum did not decrease significantly.

Khan (2005) studied therapeutic value of safflower petals decoction in hypertensive diabetic and multiple health problems subjects. The decoction of various concentrations (1.0, 1.5 and 2.0 per cent) of safflower petals

administered for a period of 0, 30 and 60 days to selected subjects. From the findings it was concluded that the intake of safflower petal decoction by the hypertensive subjects was found to reduce significantly the blood pressure when compared with their initial values. The intake of 1.0 and 1.5 per cent concentration of safflower petals decoction for 60 days had reduced the fasting serum glucose level of hypertensive subjects. Results revealed that administration of safflower petals decoction had exerted marked influence on serum lipid profile of the subjects. Hence the administration of safflower petal decoction showed beneficial therapeutic effect on selected subjects.

Ingole (2007) prepared the sesamum chutney Niger seed chutney and linseed chutney by incorporation of 20% safflower petals powder. Among prepared chutney linseed chutney was administered to hypertensive subjects for a period of 60 days. Findings of study indicate that administration of linseed chutney to hypertensive subjects reduced significantly blood pressure when compared with initial values. It was also noticed that there was slight reduction in the lipid profile values.

2.3.4. Studies on therapeutic value of tulsi (*Ocumum sanctum*)

Javanmardi *et.al* (2003) reported that India basil possesses value able antioxident properties for culinary and possible medicinal use.

Naresh *et al.* (2010) studied the comparative effect of *Ocimum sanctum*, commiphora mukul, folic acid and ramipril on lipid peroxidation in experimentally induced hyperlipidemia and found that treatment with c. mukul and O. sanctum showed significant decrease in cholesterol and triglyceride levels. O. sanctum also significantly increase serum HDL cholesterol C.mukul and *O. sanctum* had beneficial effect on hypercholosterolemic rabbit model both in terms of lipid profile as well as antioxidant potential. *Ocimum sactum* was found to be the most promising of all the drugs.

Sakar *et.al* (1994) found that treatment with 1-2 g % of os fresh leaves in the diet for four weeks significantly decreased the serum lipid profile in normal albino rabbits.

Suanarusaswat and songs (2005) found that supplementation of dried 0.5 leaf power in the diet suppressed the high serum lipid profile in diabetic rats.

Rai *et.al* (1996) conducted a study that the tulsi leaf powder was fed at the 1 percent level in normal and diabetic rats for a period of one month to explore the effect of fasting blood sugar and the lipid profile in serum and tissue lipids. The results indicated a significant reduction fasting blood sugar, total cholesterol, triglycerides, phospholipids and total lipids. In liver, total cholesterol, triglycerides and total lipids were significantly reduced in kidney. In heart, a significant fall in total cholesterol and phospholipids was observed. All these observations indicate the hypoglycaemic and hypolipidemic effect of tulsi in diabetic rats.

Anita *et.al* (2008) studied the effect of supplementation of tulsi and neem leaves on blood glucose and serum lipid profile of non insulin dependent diabetics. Supplementation of these medicinal plants was carried out for a period of three months. Daily dosage of 2 gm powder in the form of four capsule of 500 mg capacity each was given along with lunch and dinner. They found significant decrease in both fasting and postprandial blood glucose level of subjects after supplementation. Also it is observed that triglyceride, LDL – C and VLDL – C levels of subjects were reduced significantly. But non-significant change in HDL cholesterol level is observed. In conclusion, the results showed that supplementation with leaf of tulsi, neem and with mixture of both up to 2 gm daily in the form of capsule to diabetic patients helped in the reduction of their blood glucose and lipid profile.

Shweta et al (2006) investigated the antihyperlipidemic and antioxidant effect of *Ocimum sanctum* Linn. seed oil (OSSO) (0.8 g/kg body weight / body) feel rabbits significantly decreased serum cholesterol 51%, triglyceride 47% and LDL + VLDL cholesterol 59% as compared to untreated cholesterol fed group. In conclusion results of study demonstrate that osso has significant by hypolipidemic and antioxidant activity. lipid lowering effect may be to some constituent in oil which either increase catabolism or interfere with absorption of cholesterol. The antitoxicant effect of osso may be related to its hypercholesterolemic property.

The plant has been studied for their various pharmacological activities like antioxidants, antibacterial, antifungal, hypoglycemic and hypolipidemic activity. Therefore it is necessary to exploits its maximum potential in the field of medicinal and pharmaceutical sciences in novels for fruitful application.

From all the above studies it can be concluded that vegetables are rich source of vitamins, minerals and phytochemicals which prevents diseases, functioning as dietary fiber, antioxidant, detoxifiers, immunopotenting agents, having important role in preventing chronic diseases like heart disease and hypercholesterolemia. Due to great medicinal value and nutritive value herbs are processed into different food item such as candy, jam, pickles, also used in cookery as preservative aromatic and flavouring compounds.

MATERIAL AND METHODS

Chapter III

MATERIALS AND METHODS

The present investigation was carried out in two phases. In first phase of experiment three variation of Herbomix were developed utilizing selected herbal foods i.e amla, bottle gourd, safflower petals, tulsi leaves. The developed variations of herbomix were evaluated for organoleptic characteristics further highly accepted herbomix was analysed for nutrient content and used for supplementation.

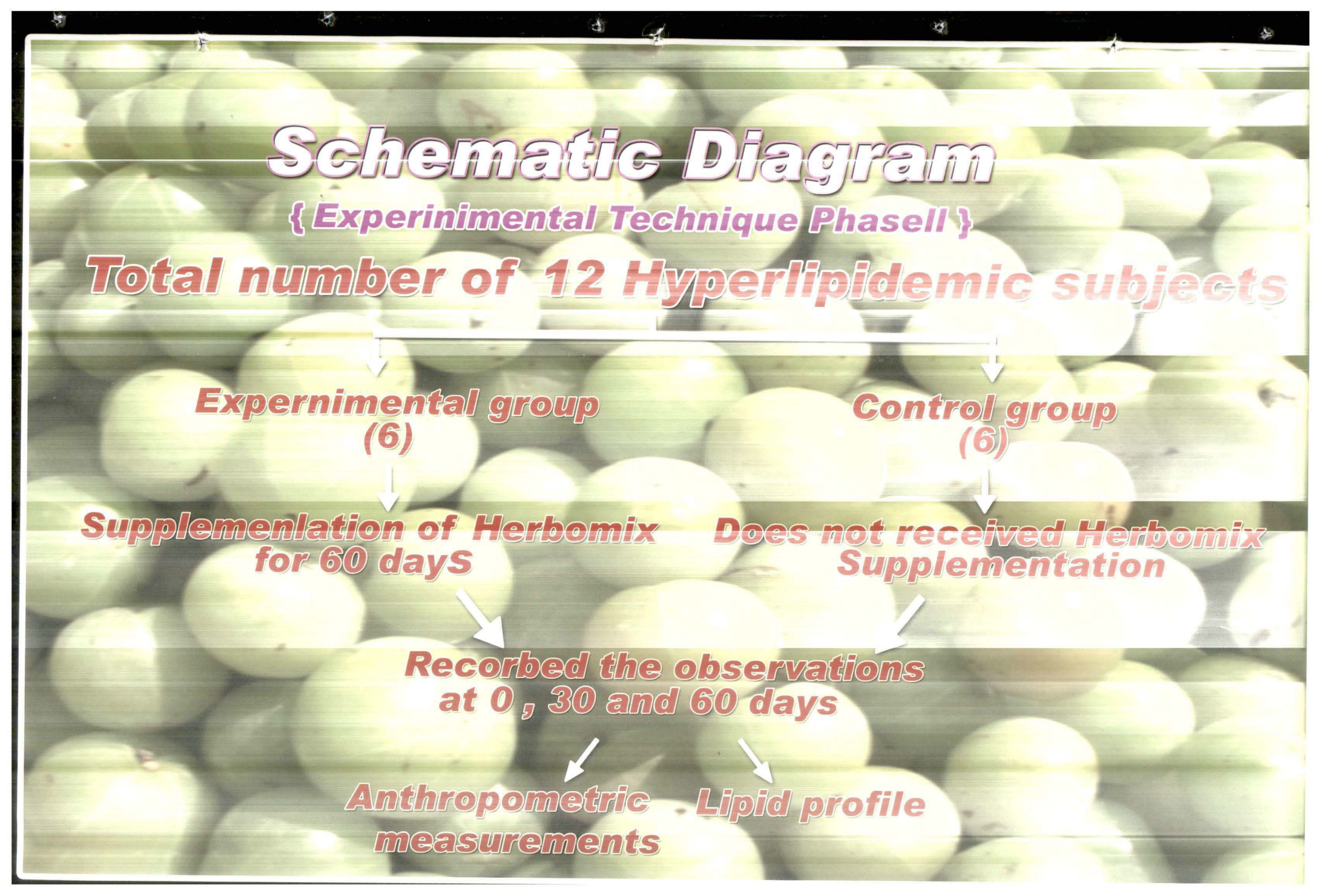
In second phase of experiment human studies were conducted. Subjects having high lipid profile were selected following the purposive sampling technique. The information regarding socio-economic background, health status and food consumption pattern was collected by personal interview method using pre-tested questionnaire. The developed herbomix was supplemented to the selected subjects for 60 days and effect of supplementation on anthropometric measurements, and lipid profile was determined.

3.1 Selection of herbal foods

Herbal foods having the medicinal and curative properties were selected for present study. The selected herbal foods were as follows.

Selected herbal foods

Sr. No	Name of the herbal food	Botanical Name
1	Amla	*Emblica officinalis*
2	Bottle gourd	*Lagenaria siceraria*
3	Safflower	*Carthamus tinctorius*
4	Tulsi leaves	*Ocimum sanctum*


Schematic Diagram
{ Experinimental Technique Phasell }
Total number of 12 Hyperlipidemic subjects
Expernimental group (6)
Control group (6)
Supplemenlation of Herbomix for 60 dayS
Does not received Herbomix Supplementation
Recorbed the observations at 0 , 30 and 60 days
Anthropometric measurements
Lipid profile

Ingredients used in Herbomix

Extraction of Bottle gourd Juice

3.2 Collection of herbal foods

Amla, bottle gourd, tulsi leaves were collected from the local market/ surrounding and safflower petals were collected from All India Co-ordinate Research Project, College of Agriculture, M.A.U., Parbhani.

3.3 Preparation of Herbal foods powder/ juice

Safflower petals and tulsi leaves were cleaned, made free form waste materials, dried in the shade and fine powder was prepared in laboratory mixer. While amla was dried in mechanical drier and powder was prepared in laboratory mixer. These herbal foods powders were stored in airtight plastic container till the end of experiment fresh amla and bottle gourd were collected, washed, trimmed and crushed in laboratory mixer and juice was extracted through muslin cloth. The juice was prepared fortnightly during the experimental period.

3.4 Development of Herbomix

Three variations of Herbomix were developed by utilizing amla, safflower petals powder, tulsi leaves powder in varying amount while the constant amount of amla juice, bottle gourd juice, honey and jaggry was used in all three variations of herbomix. The detail description of development of herbomix is as follow.

3.5 Preparation of Herbomix

Measured quantity of amla and bottle gourd juice were mixed and in this safflower petals powder, tulsi and amla powders were added and prepared the mixture. Further jaggary was added and cooked till required consistency. Prepared herbomix was cooled, added honey and mixed well.

3.6 Sensory evaluation

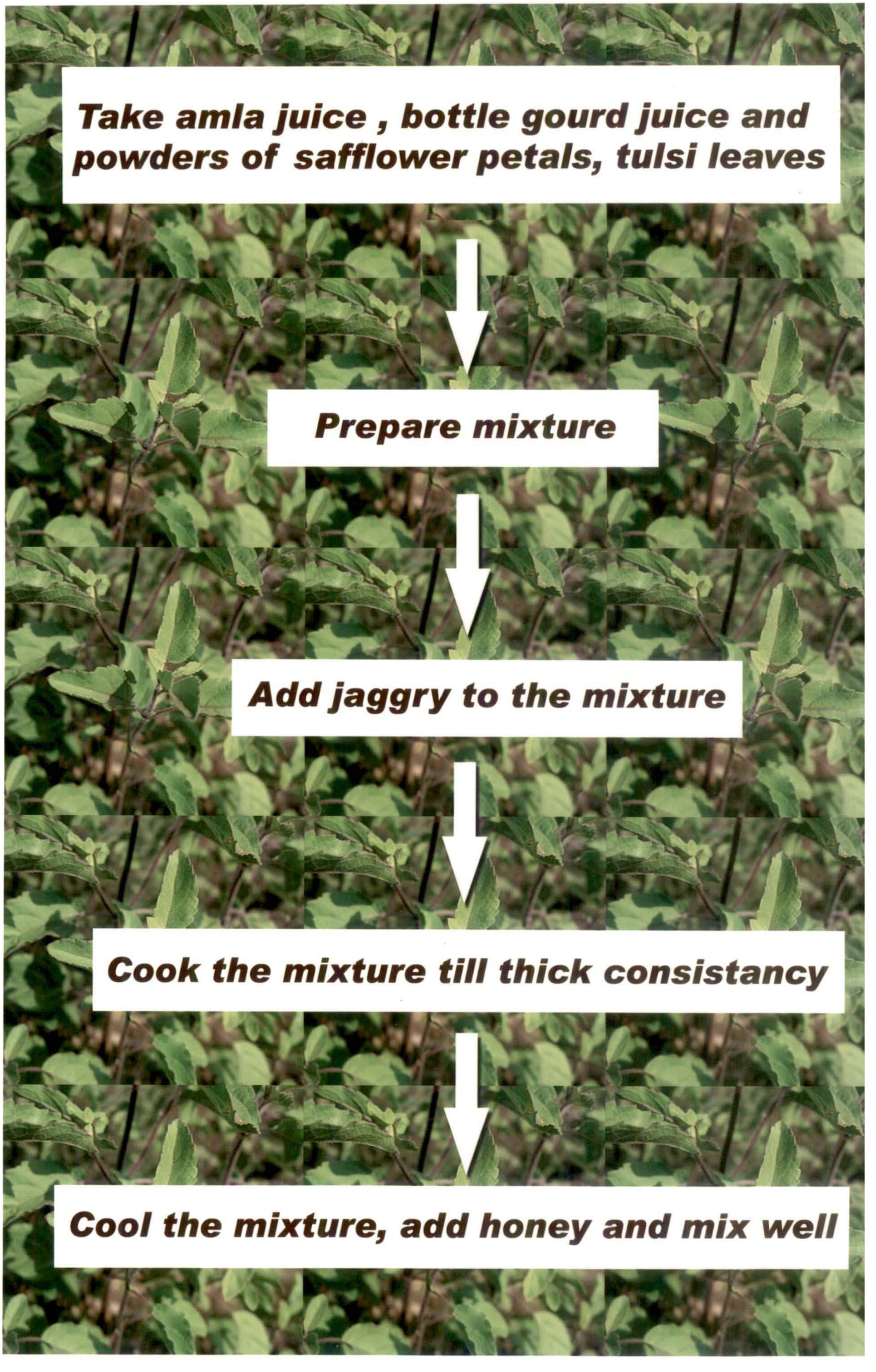
Evaluation of herbomix for acceptability test was carried out following ranking method (Ranganna, 1979) the variations of herbomix were served to ten trained judges to score for different sensory characters namely colour, texture, taste, flavour and overall acceptability at room temperature

Description of Development of Herbomix

Herbomix Variations	Description of ingredients used						
	AP (g)	AJ (ml)	BJ (ml)	S (g)	T (g)	J (g)	H (g)
Variation I	1.0	5.0	15.0	0.5	0.5	5.0	5.0
Variation II	1.5	5.0	15.0	1.0	1.0	5.0	5.0
Variation III	2.0	5.0	15.0	1.5	1.5	5.0	5.0

AP - Amla powder
AJ - Amla juice
BJ- Bottle gourd juice
S - Safflower petals powder
T - Tulsi leaves powder
J - Jaggry

H - Honey



Flow Chart of preparation of Herbomix

with the help of score card (Appendix I).Among the variations the highly accepted herbomix was selected for supplementation.

3.7 Nutrient analysis

The nutrient content of highly accepted variation of herbomix was analyzed. Moisture free sample was used for analysis. The proximate composition (moisture, total protein, fat, fiber and total minerals)Was carried out as per procedures prescribed by A.O.A.C(1975). Carbohydrate content was calculated by difference method. Calcium was estimated by

EDTA method. Trace elements (iron, copper, zinc and manganese) were estimated by Atomic Absorption Spectrophotometer (Perkin R. Elmer Model-3110)

The values for all nutrients were averages of triplicate value on dry weight basis. Moisture was expressed on fresh weight basis.

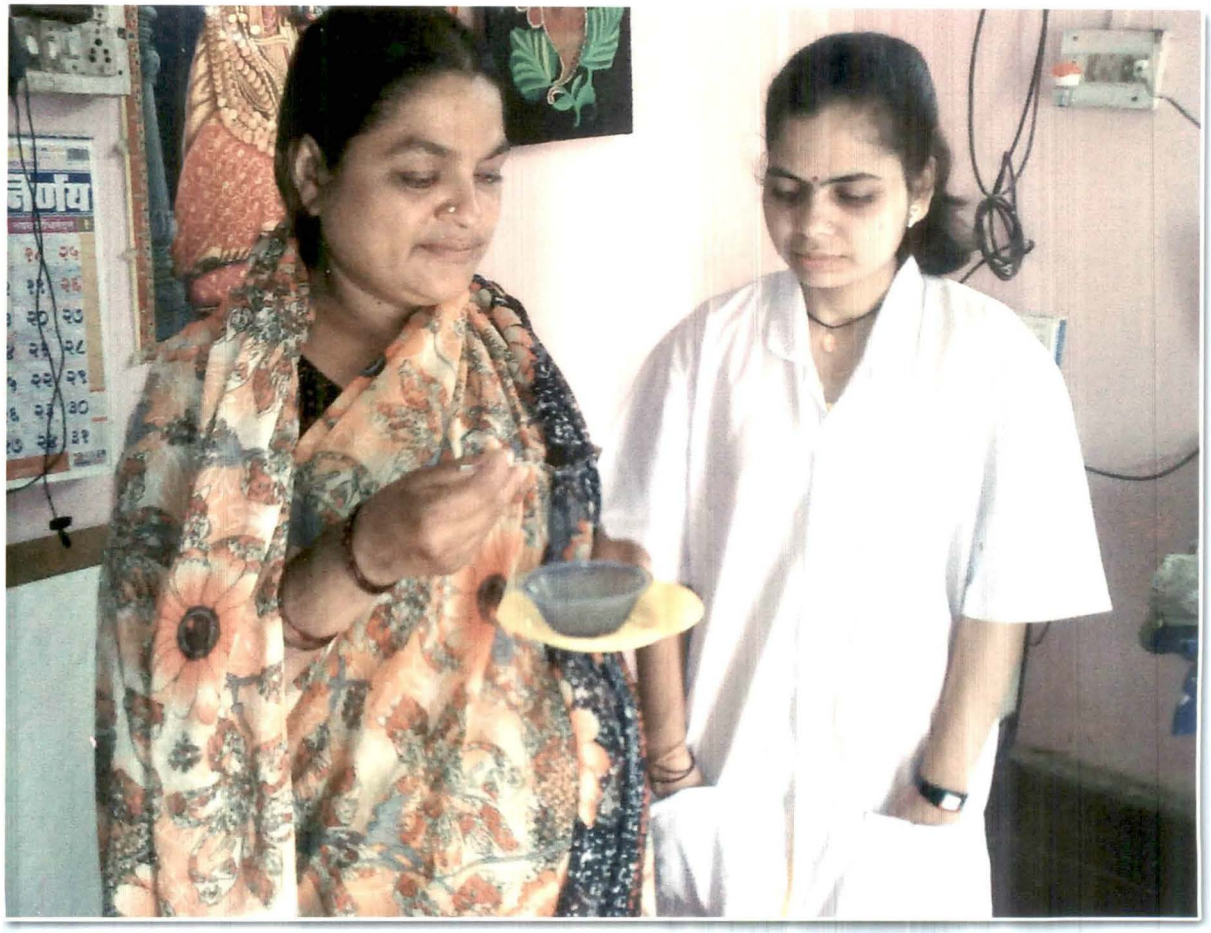
3.8 Selection of subjects for the study

A purposive sampling technique was followed to select subjects. A total number of twelve subjects suffering from hyperlipidemia volunteered to co-operate were purposively selected for the present study. Among the twelve subjects six subjects were treated as experimental group and remaining six were as control group. The purpose and discipline involved in the study was explained to all the subjects. During the study period, the selected subjects were allowed to continue and follow their usual living style, diet, exercise and medicines.

3.9 Collection of general information of selected subjects

Twelve selected subjects were personally interviewed by the investigator with the help of pretested questionnaire (Appendix II) so as to elicit the information regarding socioeconomic background, age, sex, food habits, duration of disease and presence of any other diseases.

Preparation of Herbomix



Subject consuming Herbomix

3.9.1 Diet survey of selected subjects

The information regarding food consumption pattern of selected subjects was obtained by personal interview method. The 24 hour recall method was used to asses the food and nutrient intake of selected subjects. The intake of the food in cooked form was converted into raw food ingredients and the nutritive value of the raw foods was determined to find out the nutrient intake of the subjects per day, following the Nutritive Value of Indian Foods. The mean nutrient intake of subjects was compared with Recommended Dietary Allowance of ICMR (2000).

3.10 Experimental technique

In the second phase of the study, a total number of 12 selected subjects suffering from hyperlipidemia were divided into two groups viz. experimental group and control group, each group consisted of six subjects. The experimental group was given highly accepted herbomix supplementation every day for a period of 60 days in addition to their daily routine diet where as control group was not receiving the herbomix but following their daily routine diet.

Freshly prepared herbomix was supplemented to the selected subjects daily in the morning hours i.e. around 9.00 am in an amount of 20 gm to each subject for a period of 60 days and periodic observations at 0, 30 and 60 days were recorded of experimental and control group.

3.11 Clinical Study

The anthropometric measurements Viz. weight, height, mid arm circumference, tricep skin fold and body mass index, and biochemical test like serum lipid profile i.e. triglycerides, total cholesterol, LDL and HDL cholesterol level of control and experimental were recorded initially and after 30^{th} and 60^{th} day of experimental period following standard procedures.

3.12 Recording anthropometric measurement

The body measurements of the selected subjects recorded during the experimental period were weight (kg), height (cm), mid arm circumference

(cm) and tricep skin fold thickness (mm) by the following the standard procedures described by Jelliffe (1966) and body mass index (BMI) was calculated by using the standard formula. Details of the procedure followed and materials used for measuring different anthropometric indices are described here under.

3.12.1 Determination of body weight of the selected subjects

A portable weighing balance of 100kg capacity with a sensitivity of 0.1 kg was used to record the weigh of the each one of the selected subjects. The body weight noted to the nearest 0.1 kg when the subject was standing upright on the balance with bare feet and light indoor clothing. This procedure was repeated for two times and the mean value of the weight (kg) was taken as a measurement of body weight of the subjects.

3.12.2 Determination of body height of the selected subjects

The height of the selected subjects was measured by using no stretchable measuring tape. The subject was asked to stand upright with bare feet close tighter, less straight, arms at the sides and shoulder relaxed against the wall. Cardboard was placed perpendicular to the crown of the head of the subject. Then, a mark was made on the wall with the help of a pencil. The actual height of the subject was measured with the help of a measuring tape to the nearest 0.1 cm by measuring the length from the mark to the floor.

3.12.3 Determination of body mass index of the selected subjects

Body mass index (BMI) was calculated from the recorded measurement of body weight (kg) and height (cm) of the subjects using the following formula

$$\text{Body Mass Index} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

Mesurement of weight

Mesurement of height

3.12.4 Determination of tricep skin fold thickness of the selected subjects

Skin fold thickness at tricep was measured by using skin fold caliper. The measurement of skin fold thickness at triceps was made at midpoint on the back of the left arm over the tricep muscle. The skin fold thickness was recorded to the nearest 0.1 mm while the jaws of the caliper were placed perpendicular to the length of the skin fold at the marked midpoint of left arm. This procedure was repeated twice and means value was taken as the measure of skin fold thickness at triceps.

3.12.5 Determination of mid arm circumference of the selected subjects

The circumference of the left upper arm was measured at its midpoint for the determination of mid-arm circumference of the selected subjects. Midpoint of the arm was marked by keeping the left forearm at 90^{0} at the Point of elbow and by placing palm facing down, across the middle of the body .Care was taken to keep the upper arm approximately parallel to the trunk .The tape was used around the arm and the measurement was recorded twice at the marked midpoint to the nearest 0.1 cm, and the mean value was taken as the measure of mid-arm circumference.

All the above mentioned anthropometric measurements were recorded on the day of starting of the experiment and also on 30^{th} and 60^{th} days of study period.

3.13 Analytical techniques

About two ml of blood was taken from antecubital vein of each subject with the help of trained around 8.00 am. The initial blood sample was collected before starting the study and after the administration of Herbomix supplementation with periodical interval of 30 and 60 days.

The collected blood samples were analysed to find the fasting serum lipid profile following at the standard methods. The collected blood was allowed to clot then serum was separated from the blood clot as rapidly as

possible by the centrifugation. The serum thus obtained was used as a sample for the estimation of lipid profile.

3.13.1 Estimation of cholesterol in serum by CHOD-PAP method (Schettler *et al.* 1975)

3.13.1.1 Principle

The total cholesterol in serum is determined after enzymatic hydrolysis and oxidation. The indicator quinomeimine is formed from hydrogen peroxide and 4 aminophenazone in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + 0_2 \xrightarrow{\text{CHO}} \text{Cholesterol} - 3 - \text{One} + H_2O$$

$$2H_2O + 4 \text{ amino phenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinone amine} + 4H_2O_2$$

3.13.1.2 Reagents

A kit purchased from Stand Diagnostics Ltd., Mumbai had the following reagents for ready to use purpose to determine the cholesterol in the serum.

3.13.1.2.1 Enzyme reagent

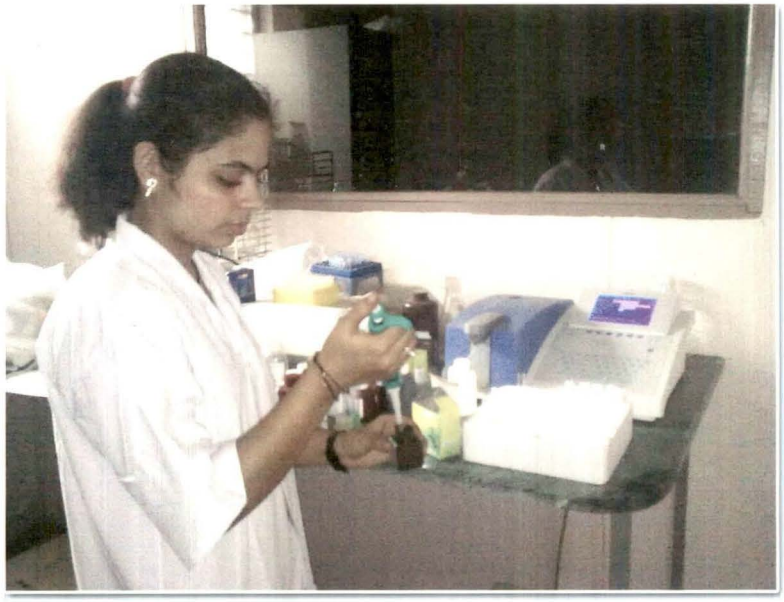
This reagent contained phosphate buffer (pH 6.5) (100 mmol/lit), 4-aminophenazone (0.25 mmol/lit), phenol (5 mmol/lit) peroxidate (>5 ku/I), cholesterol esterase (>150 U/I) and cholesterol oxidase (>100 U/I).

3.1.1.2.2 Cholesterol standard

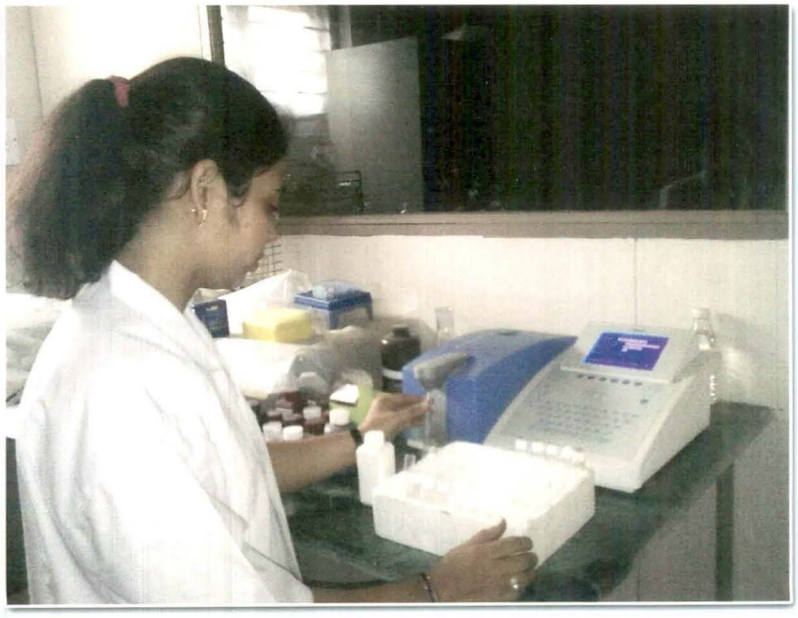
This standard solution had 200 mg of cholesterol in 100 ml solution.

3.13.1.2.3 Procedure

As per the details given in the table, the solutions were mixed well and incubated for 5.0 minutes at 37^0C.



Preparation of samples



Analysis of serum lipid profile

Pipette into curvettes	Reagent blank	Sample of standard
Sample/ standard	--	10 µl
Reagent	1000 µl	1000 µl

The absorbance of the sample/standard was read in the spectrophotometer (TRANCESIA) at 500 nm, within 30 minutes against the reagent blank (AA).

3.13.1.3 Calculation

Cholesterol concentration mg/dl = 553 x AA

3.13.2 Estimation of triglycerides in serum

The level of triglycerides in serum was estimated by following enzymatic colorimetric method GPO-PAP as described by Bucolo *et al.* (1973)

3.13.2.1 Principle

The principle of determination of triglycerides is based on the following reactions.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol – 3 – phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate}$$

$$H_2O_2 + \text{4AAP + 4 cholesterol} \xrightarrow{\text{POD}} \text{Quinoneimine + } H_2O$$

3.13.2.2 Reagents

All the reagents were obtained as components inkit purchased from Stand Diagnostics Ltd., Mumbai for ready to use purpose.

3.13.2.2.1 Single reagent

The reagent was stored at 2-80c and it was brought again to room temperature prior to the usage.

3.13.2.2.2 Standard solution

This solution had concentration of 200 mg of triglyceroides per 100 ml

3.13.2.2.3 Procedure

As per the details given in the following table the solutions were mixed and incubated at 370C for five minutes.

Pipette into cuvettes	Reagent blank	sample of standard
Sample/standard	--	20µl
Reagent	2 ml	2 ml

Absorbance of the standard (As) and samples (Av) were recorded at 510 nm against reagent blank within 30 minutes in spectrophotometer (TRANCESIA).

3.13.2.4 Calculation

$$\text{Triglycerides in mg/dl} = \frac{Ax}{As} \times 200$$

3.13.3 Estimation of HDL cholesterol and LDL cholesterol

HDL cholesterol and LDL cholesterol in the serum samples were estimated by high performance CHOD-PAP method as described by Lopes *et al.* (1977).

3.13.3.1 Principle

LDL (Low density lipoprotein) cholesterol is precipitation by adding phosphotungstic acid and magnesium ions to the samples. Centrifugation leaves only the HDL (high density lipoproteins) cholesterol in the supernatant and the cholesterol content is determined enzymatically.

3.13.3.2.1 Reagents

Reagents were obtained from Stand Diagnostics Ltd., Mumbai in the form of a kit for ready to use purpose.

3.13.3.2.1 Precipitant reagent

It contained phosphotungstic acid 0.55 nmol/1 and magnesium chloride 25 nmol/l this reagent was stored at 15^0c to 25^0c for the use.

3.13.3.3 Procedure

Pipette into centrifuge tube	Macro
Sample	500 µl
precipitation	100 µl

The serum sample and the precipitation reagent were mixed and allowed to stand for 10 min at room temperature. This contents were centrifuged for 10 min at 4000 rpm. The clear supernatant was separated with in two hours and for the cholesterol was determined by CHOD-PAP method as described in this chapter under the estimation of total cholesterol.

3.13.3.4 Calculation of HDL cholesterol

HDL cholesterol in mg/100 ml = 187.9 x absorbance of sample.

3.13.3.5 Calculation of LDL cholesterol

$$\text{LDL-cholesterol (mg/100 ml)} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol (mg/100 ml)}$$

3.14 Statistical analysis

The data of acceptability of four developed variations of herbal composite were statistically analysed by one way analysis of variance and 'F' values were calculated to find out the difference among the developed

variations of herbal composite prepared with varying amount of honey and jaggery.

To determine the impact of supplementation of herbal composite at 0, 30 and 60 days of experiment on anthropometric measurements, blood pressure and serum lipid profile of selected subjects the means, range and standard error of different parameters were calculated.

The differences with regard to values between 0,30 and 60 days were of experimental and control group and also the difference between initial and final values of experimental Vs control was compared by applying 't' test (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present study was designed in two phases, In first phase of experiment three variations of Herbomix were prepared by utilising selected herbal foods viz. amla, bottle gourd, safflower petals, and tulsi. Developed herbomix were evaluated by panel members for acceptability in terms of organoleptic characteristics.

In second phase human studies were conducted in order to evaluate the effect of supplementation of herbomix on selected subjects.

The data was tabulated statistically analysed and discussed here under various heads.

4.1 Organoleptic characteristics of herbomix developed by utilising selected herbal foods

The mean values of organoleptic scores for the acceptability of herbomix developed by utilising selected herbal foods, amla, bottle gourd, safflower petals and tulsi are give in Table 1 and illustrated in Figure 1.

The mean scores for colour of I, II and II variations of Herbomix were found to be 4.3, 4.3 and 3.5 respectively. Variation I and II secured highest score (4.3) than that of variation III (3.5).

Statistical analysis revealed that the scores obtained for the colour of herbomix variation I and II differed significantly with variation III.

It can be said that among prepared variations of herbomix variation I and II was found to be most accepted with regard to colour.

Table 1 Mean acceptability scores of organoleptic characteristics of developed Herbomix (n = 3)

Herbomix variations	Colour	Texture	Flavor	Taste	Overall acceptability
I	4.3	4.3	4.2	4.3	4.4
II	4.3	4.0	3.9	4.1	4.1
III	3.5	3.5	3.2	3.1	3.2
Mean	4.0	3.9	3.7	3.8	3.9
F value	6.5*	4.8NS	20.8**	14.2**	41.2**
SE	0.16	0.18	0.10	0.17	0.09
CD	0.56	0.62	0.35	0.59	0.31

** Significant at 1% level

* Significant at 5% level

NS Non significant

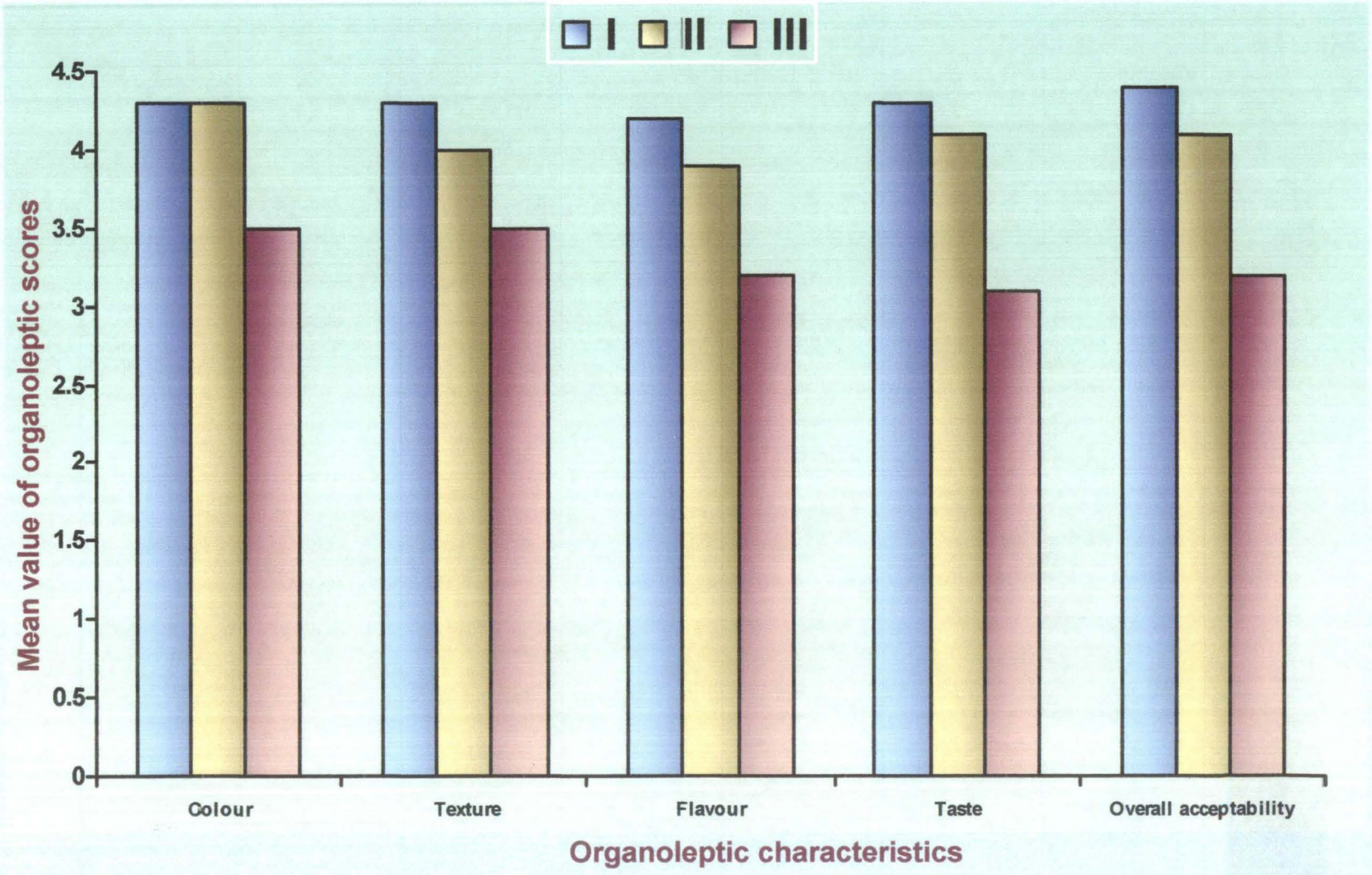


Fig 1 Mean acceptability scores of organoleptic characteristics of developed herbomix

The scores recorded for the texture of herbomix prepared by utilising selected herbal foods were between 3.5 to 4.3. The maximum score was recorded by variation I and the minimum score was recorded by variation III.

Statistical analysis revealed that the scores obtained for texture of variation I differed significantly with variation III. From the results it can be concluded that variation I was found to be the most acceptable in the context of texture.

The mean scores secured for flavour of herbomix was ranging from 3.2 to 4.2. Variation I secured highest score of 4.2 whereas minimum score of 3.2 was secured by variation III.

Statistical significant difference for flavour between variation I and III in also in variation II and III was recorded.

On the whole, it can be concluded that variation I was most acceptable in terms of flavour.

The scores registered for the taste were 4.3, 4.1 and 3.1 for variation I, II and III respectively. The highest score of 4.3 was recorded by variation I whereas, lowest score of 3.1 was secured by variation III.

On the whole it can be said that the variation I was found to be highly acceptable in terms of taste.

The mean scores for overall acceptability of herbomix were ranging from 3.2 to 4.4. The maximum score was obtained by variation I (4.4) followed by variation II (4.1) and variation, III (3.2).

Statistical result showed that overall acceptability of herbomix of variation I and II differed highly significant than that of variation III.

From results it can be said that the highest score for overall acceptability was recorded by variation III.

In conclusion of above results it can be said that variation I was found to be most acceptable in terms of all organoleptic characteristics.

Mamila (2009) prepared four variations of herbal composite utilising amla, safflower, curry leaves, tulsi, mint and evaluated for organoleptic charactersitcs. The results of sensory evaluation revealed that among developed variations the variation IV was found to be most acceptable in terms of all organoleptic characteristics.

4.2 Nutritional composition of herbomix

Three variations of herbomix were developed. Among developed herbomix variation I recorded highest scores for organoletptic characteristics. Thus the nutrient content of Variation I was analysed. Proximate Composition of herbomix is presented in Table 2.

The contents of moisture (g%), protein (g%), fat (g%), total mineral (g%), crude fibre (g%) and CHO (g%) in herbomix were 27.41, 3.2, 0.4, 2.60, 2.85 and 63.54 percent respectively. The results of the study revealed that the herbomix contained more amount of total minerals 2.6 g/100gm fibre 2.85 g/100gm and low level of fat 0.4 g/100gm.

Nutrients other than fat play an important role in preventing or promoting atherosclerosis by changing blood levels of cholesterol, HDL cholesterol and triglyceride.

In recent years, there has been revival of interest in the nutritional aspect of fat, as excessive intake of fat in diet increases the level of cholesterol in blood, which gradually leads for deposition under the lining of blood vessels.

Cardio vascular disease is less common in population that have high fibre diets than it is in populations in developed countries where a more refined lower fibre diet is consumed.

Fibre may influence blood lipid levels through their capacity to bind bile salts and cholesterol, preventing their absorption, fibre forms a gel in the intestine, which results in the binding of bile acids, an important ingredient among the juices in the body. When an insufficient amount of bile acid reach

to liver the liver converts the cholesterol into bile acid there fore help to reduce cholesterol level.

Minerals are important for vital body functions such as acid base and water balance vegetables contribute these minerals and enhance their availability in daily life. Adequate amount of supply of trace elements in the diets is of great current interest of nutrition of community because increasing evidence of their marginal or inadequate intake among different segments of population (Weaver *et al.* 1981).

Trace elements contents (g/100gm) of calcium (mg/100 gm), iron (mg/100 gm), copper (mg/100 gm), zinc (mg/100 gm) and manganese (g/100 gm) of the herbomix are presented in Table 3.

The estimated value for calcium, iron, copper, zinc and manganese were 282.00, 10.2, 10.41, 18.16, and 7.3 mg/100 gm respectively.

Calcium is an essential element for several life processes. It is required for normal contraction of muscle to make limbs move, contraction of heart for its normal function, nervous activity and blood clotting (Revanwar, 1996).

Like vitamins, minerals cannot be synthesized by the human body and hence must be provided in diet. Minerals are necessary, for the regulatory systems in the body for the efficient metabolism of proteins, carbohydrates, fats and for other regulatory functions. But trace elements are found to be deficient in the diet of population in developing countries (Schroeder, 1997).

Table 2 Proximate composition of herbomix

Nutrients	(g%)
Moisture	27.41
Protein	3.2
Fat	0.45
Total mineral	2.60
Crude fibre	2.85
Carbohydrate	63.54

Table 3 Trace elements contents of herbomix

Nutrients	(g%)
Calcium	282.00
Iron	10.2
Copper	10.41
Zinc	18.16
Manganese	7.3

A large number of minerals and trace elements are present in blood. Some of these form part of body structural component and some others act as catalytic agents in many body reactions.

On the whole, it can be concluded from the results that the development of herbomix by utilizing selected herbal foods provides more amount of nutrients like crude fibre, total minerals, calcium, iron and good amount of zinc and low fat content which is beneficial for hyperlipidemic subjects.

Herbs are source of active principles having antioxidant property and chemicals like phenols, polyphones and flavonoids, which play an important role in preventing diseases. Hence herbomix can be suggested for the consumption from therapeutic point of view as it contains phytochemical and the fibre rich foods are usually low in carbohydrate and are important for decreasing the risk of diseases like Parkinson`s disease, Alzheimer's disease, cardiovascular diseases, cell apoptosis and even cancer also.

4.3 Socio economic background and health status of the selected subjects

The data on socio economic background and health status of 12 selected subjects are presented in table 4.

The general information of selected subjects indicated that all the selected subjects were above age of 30 years.

Among the selected subjects 83 per cent were female and 17 per cent were male.

It is clear from results that 67 per cent subjects had income between five to ten thousands , 25 per cent were found to have monthly income more than 10000-15000 and remaining were having monthly income more than 15000.

Maximum number (11) of the selected subjects were form nuclear families while only one was from joint family.

The selected subjects were having qualification of primary, high school and college level were 25, 50 and 25 per cent respectively.

Majority of selected subjects (66%) were homemaker followed by government servants (17%) and businessman (17%).

Table 4. Socio economic background and health status of the selected subjects.

Attributes	No. of subjects (n = 12)	Per cent
Age in years		
Age > 30	12	100
Sex		
Male (2)	2	17.00
Female (10)	10	83.00
Monthly income		
5000 - 10000	8	67.00
> 10000 – 15000	3	25.00
> 15000	1	8
Type of family		
Nuclear	11	92.00
Joint	1	8.0
Education		
Primary	3	25.0
High school	6	50
College	3	25
Occupation		
Home maker	8	66.00
Government servant	2	17.00
Business	2	17.00
Meal patterns		
Two	3	25
Three	9	75
Food habit		
Vegetarian	9	75
Non vegetarian	3	25
Duration of disease		
1 – 5 years	2	17
> 5 years	10	83
Kind of medicine		
Allopathy	8	67
Homeopathy	--	--
Ayurvedic	1	8
No medicine	3	25

Among selected subjects majority number of subjects (75%) followed three meal pattern while only 25 per cent follow two meal pattern. 75 per cent subjects were found to be vegetarian and 25 percent were non vegetarian.

Among the selected of subjects maximum number of subjects (10 were having the history of disease of more than 5 years while minimum number of subjects (2) were having history of disease from 1 – 5 years. Among these subjects majority of subjects (8) were taking allopathy, while only one was taking ayurvedic medicines and remaining three were not taking any type of medicines.

In a nut shell it can be concluded that most of the subjects were belonging to middle income group nuclear family. Moderately educated and had the history of disease more than five years and most of them were taking allopathic medicine.

4.4 Assessment of dietary and nutritional intake

4.4.1 Adequacy in the intake of different foods per day by the experimental group

Per cent of adequacy in the intake of different foods per day by the experimental group is presented in table 5.

The value of adequacy (per cent) in the intake of cereals experimental group was observe as 67.55. Adequacy values in intake of pulses other vegetable, milk and milk products and fats and oils was more than 100. Among all these highest adequacy value (140) was observed for fats and oils. On other hand adequacy value in intake of green leafy vegetables was less than 20 per cent. Adequacy values in intake of fruits, Roots and tuber and sugar were 62.5, 73 and 56.25 respectively. From the results it can be concluded that diet consumed by experimental group was more than adequate in supplying the pulses, other vegetables, milk and milk products and fats and oils. Whereas intake of green vegetables by experimental group was very less. The results also showed that the diet was inadequate in supply of food stuffs like cereals, fruits and roots and tubers.

4.4.2 Adequacy in the intake of different foods per day by the control group

Per cent of adequacy in the intake of different foods per day by the control group is presented in table 6.

Per cent adequacy for cereals, pulses, green leafy vegetables, roots and tubers and milk and milk products by male respondents of control group was found to be in range of 53.33 to 76.00 per cent. The adequacy values for other vegetables and sugar and jaggery was nearer to RDA as 93.33 and 100 per cent respectively. On the other hand fats and oils per cent adequacy was more than RDA as 170 per cent.

In case of female respondent of control group the intake of cereals, pulses and milk and milk products was in between 71 to 80 per cent. The adequacy values for other vegetables roots and tubers and sugar were found to be nearer to RDA's. Minimum adequacy value was observed for intake of green leafy vegetable as 26 per cent while adequacy value for fats and oils was higher than RDA as 150 per cent.

Table 5. Adequacy in the intake of different foods per day by the experimental group (n = 6)

Food stuffs (g)	Mean intake (Female)	Required intake	% Adequacy
Cereals	202.5	300	67.2
Pulses	47.5	40	118.75
Fruits	62.5	100	62.5
GLV	15	100	15
Other vegetables	159.3	50	118.6
Roots and tubes	36.5	50	73
Milk and milk products	225	200	112.2
Sugar and jaggery	11.25	20	56.25
Fats and oils	28	20	140

Table 6. Adequacy in the intake of different foods per day by the control group (n=6)

Foods stuffs (g)	Mean intake (Male)	Required intake	% Adeq-uacy	Mean intake (Female)	Required intake	% Adeq-uacy
Cereals	275	400	68.75	213	300	71
Pulses	38	50	76	32	40	80
Fruits	75	100	75	--	100	--
Green leafy vegetables	45	75	60	26	100	26
Other vegetables	70	75	93.33	50	50	100
Roots and tubers	40	75	53.33	49	50	98
Milk and milk products	150	200	75	150	200	75
Sugar and jiggery	20	20	100	20	20	100
Fats and oils	34	20	170	20	20	150

On the whole per cent adequacy in male and female respondents in regard to fats and oils is more than requirement while values for adequacy of other vegetables roots and tubers and sugar and jaggery were nearer to RDAs whereas diet was found to be inadequate in intake of cereals, pulses, fruits green leafy vegetable milk and milk products in both groups.

4.4.3 Adequacy in the intake of different nutrients per day by the experimental group

Per cent of adequacy in the intake of different nutrients per day by the experimental group is presented in Table 7.

It is clear from results that maximum adequacy values were observed for consumption of fat 233.13 per cent followed by calcium 133. 25 per cent where as fiber recorded minimum adequacy 13.4 per cent. Per cent adequacy values for energy protein and carbohydrates were found to be 79.75, 86.93 and 70.25 per cent respectively. Whereas adequacy value for iron was less than 50.

From the results it can be said that the intake of energy protein, CHO, iron and fiber were found to be less but it was vice versa with regard to per cent of adequacy in the intake of fat and calcium. Large amount of calcium in diet lower cholesterol and triglyceride.

On the whole consumption of all nutrients except fat and calcium were not satisfactory by the experimental group in comparison to RDA's.

Table 7. **Adequacy in the intake of different nutrients per day by the experimental group (n = 6)**

Nutrients	Mean intake (Female)	RDA	% Adequacy
Energy (Kcal)	1495.37	1875	79.75
Protein (gm)	43.46	50	86.93
Fat (gm)	46.62	20	233.13
CHO (gm)	238.86	340	70.25
Fibre (gm)	5.36	40	13.4
Calcium (mg)	533.03	400	133.25
Iron (mg)	13.525	30	45.08

Table 8. **Adequacy in the intake of different nutrients per day by the control group (n = 6)**

Nutrients	Mean intake (Male)	RDA	% Adequacy	Mean intake (Female)	RDA	% Adequacy
Energy (Kcal)	1855	2425	76.49	1513	1875	80.69
Protein (gm)	47	60	78.33	37.69	50	75.38
Fat (gm)	58.81	26	294.05	42.25	20	221.85
CHO (gm)	232.43	340	95.126	217.93	340	63.82
Fibre (gm)	10.25	40	25.625	6.2	40	15.50
Calcium (mg)	613.72	400	153.43	440.00	400	110.00
Iron (mg)	15.17	28	54.17	11.39	30	37.96

4.4.4 Adequacy in the intake of different nutrients per day by the control group

Per cent of adequacy in the intake of different nutrients per day by the control group is presented in Table 8.

The per cent adequacy values of fats and oils and Calcium for male respondent was 294.05 and 153.43. The respective values for corresponding nutrients for female respondent were 221.25 and 110.00 per cent. Adequacy recorded for fats and oils and calcium were more than RDA in both respondent. Per cent adequacy in supply of energy and protein was ranging from 75 – 80 per cent in both respondent groups. Per cent adequacy value for CHO intake in male respondent was observed to be nearer to 100 as 95.12 while in case of female respondent it was only 63.82.

In case of iron per cent adequacy was observed to be 54.17 and 37.96 in male and female respondent respectively. From results it was observed that diet taken by both respondent was deficit in supply of fiber as per cent adequacy is found to be less than 30.

4.5 Anthropometric assessment.

The measurement of anthropometric indices are useful to assess the nutritional status of the individuals and thus helps to know health status of subjects.

4.5.1 Anthropometric measurements of experimental group before and after supplementation.

The mean values of anthropometric measurements of the experimental group before (0 days) and after supplementation (30 and 60 days) are presented Table 9.

The mean value of body weight of experimental group before supplementation was 58.81 $\pm$ 6.97 kg and ranged between 50-68.40 kg. Whereas, after supplementation at 30 and 60 days it was 58.03 $\pm$ 6.91 kg and 57.58 $\pm$ 6.83kg respectively.

It was observed that after 60 days of supplementation the weight of experimental group reduced by 1.23 kg. However, reduction in weight was observed to be statistically non significant ($p>0.05$).

Body weight has traditionally been used as an indicator of obesity, which raises the risk of health problems. Hubert (1983) conducted study and found a direct association of degree of obesity with CHD.

Ingole (2007) reported that administration of linseed chutney prepared with incorporation of safflower petals powder (at 2%), did not show significant effect in reducing body weight of the selected hyrpernisve and hyperlipidemic subjects.

Results of the present study are in line with results of Mamila (2009), who reported that the supplementation of herbal composite prepared by safflower petals, curry leaves, amla, tulsi and mint did not show significant effect in reducing body weight of the selected hypertensive and hyperlipidemic subjects.

The height of the experimental group was ranged from 149 to 158cm with an average value of 154.16 ± 4.95cm.

The body mass index (BMI) of experimental group was 28.81 to 3.23 initially at 0 days which reduced slightly (24.47 ± 3.17 and 24.29 ± 3.16) after 30 and 60 days of supplementation respectively. Results revealed that BMI of experimental group decreased slightly with increase in period of supplementation but statistically non significant.

BMI is a measure of relative body fatness which is used by health professional to evaluate the risk factors associated with obesity (Stark, 1987)

Anita et al (2009) reported that the supplementation of tulsi and neem in non insulin dependent male diabetics did not show significant effect in reducing body weight and body mass index of the selected subjects.

The mean value of mid arm circumference of the experimental group before supplementation was 28.10 ± 3.00cm. Whereas after 30 and 60 days of supplementation it was 27.96 ± 3.02 and 27.93 ± 2.99 cm, respectively. Results indicated that mid arm circumference of experimental group was

Table 9 Anthropometric measurements of experimental group before and after supplementation (n =6)

Lipid profile	Zero days (before)		30 days		60 days (after)	
	Range	mean ± SD	Range	mean ± SD	Range	mean ± SD
Weight (kg)	50 – 68.40	58.81 ± 6.97	49.20 -67.60	58.03 ± 6.910	49.00 – 67.00	57.58 ± 6.83
Height (cm)	149 – 158	154.16 ± 4.95	149-158	154.16 ± 4.95	149 ± 158	154.16 ± 4.95
BMI	21.28 – 28.60	24.81 ± 3.23	21.08 ± 28.24	24.47 ± 3.18	20.76 ± 27.92	24.29 ± 3.163
Mid arm circumference (cm)	23.00 – 29.50	28.10 ± 3.00	23.00 ± 29.50	27.96 ± 3.02	23.00 ± 29.30	27.93 ± 2.99
Tricep skin fold (mm)	22.00 – 42.30	30.05 ± 8.67	22.00 – 42.10	29.80 ± 8.61	22.00 – 42.10	29.65 + 8.683

't' values of anthropometric measurements (students paired 't' test)

Days	Weight (kg)	Height (cm)	BMI	Mid arm circumference (cm)	Tricep skin fold (mm)
Zero Vs 30	0.204 NS	0.117 NS	0.183 NS	0.087 NS	0.057 NS
Zero Vs 60	0.319 NS	0.117 NS	0.284 NS	0.104 NS	0.086 NS
30 Vs 60	0.115 NS	0.117 NS	0.105 NS	0.207 NS	0.034 NS

* Significant at 5% level ** Significant at 1% level

NS Non significant

decreased slightly after supplementation than that of before supplementation but statistically non significant (P>0.05).

The mean values recorded for tricep skin fold thickness of experimental group before supplementation was (30.05 ± 8.67 mm) and after supplementation of 30 and 60 days (29.80 ± 8.61mm and 29.65 ± 8.68mm) respectively.

Results showed that after 60 days of supplementation triecp skinfold thickness was reduced slightly but statistically non significant.

Body fat varies widely with individual degrees of fatness or leanness, reflecting the number and size of fat cells. Half of body fat is in the subcutaneous fat layers as insulation to maintain body temperature thus providing a useful measure in general practice, tricep skin-old for estimating body fat in relation to lean body mass.

In concussion it can be said that body weight, BMI, mid arm circumference and tricep skinfold of experimental group reduced slightly after supplementation for 60 days but statistically non significant.

4.5.2 Anthropometric measurements of experimental and control group before and after supplementation.

The changes in mean values of anthropometric measurements of experimental and control group before and after supplementation of herbomix are presented in Table 10.

The mean decrease in body weight due to supplementation in case of experimental group was 1.23 ± 0.14 and 0.27 ± 0.14 kg in control group. However comparison of the mean decrease in body weight of experimental and control group showed non significant difference. Subsequently the difference between the initial and final values were found to be statistically non significant in both groups.

Deodhar (2000), reported that the administration of 1.0 percent safflower petals decoction did not show significant effect in reducing body weight of selected hypertensive subjects.

Table 10 Anthropometric measurements of experimental and control group before and after supplementation (n=12)

Parameters	Mean ± S. D.		Difference	't' values	
	Initial (0 days)	Final (60 days)		I VSF (0 Vs 60 days)	E Vs C (60 days)
Weight (kg)					
Experimental	58.81 ± 6.97	57.58 ± 6.83	1.23 ± 0.14	0.31 ^{NS}	0.88 ^{NS}
Control	61.72 ± 6.70	61.45 ± 6.84	0.27 ± 0.14	0.062 ^{NS}	
Height (cm)					
Experimental	154.16 ± 4.95	154.16 ± 4.95	0 ± 0	-	0.12 ^{NS}
Control	154.50 ± 4.50	154.50 ± 4.50	0 ± 0	-	
BMI					
Experimental	24.81 ± 3.23	24.29 ± 3.16	0.52 ± 0.07	0.28 ^{NS}	1.80 ^{NS}
Control	26.57 ± 0.79	26.72 ± 0.77	0.13 ± 0.02	0.26 ^{NS}	
Mid arm circumference (cm)					
Experimental	28.10 ± 3.00	27.93 ± 2.99	0.17 ± 0.001	0.10 ^{NS}	1.06 ^{NS}
Control	25.37 ± 4.23	25.40 ± 4.15	6.03 ± 0.08	0.013 ^{NS}	
Tricepskinfold (mm)					
Experimental	30.05 ± 8.67	29.65 ± 8.68	0.40 ± 0.01	0.086 ^{NS}	0.02 ^{NS}
Control	29.95 ± 12.03	29.82 ± 12.13	0.13 ± 0.1	0.017 ^{NS}	

I - Initial F - Final E - Experimental C - Control
* Significant at 5% level ** Significant at 1% level. NS non significant

The average height of experimental and control group was 154.16 ± 4.95cm and 154.50 ± 4.50 cm respectively. The difference in height was not seen through out the study period.

The mean decrease in the body mass index of experimental group was found to be 0.52 ± 0.07 while in case of control group there was reduction to the tune of 0.13 ± 0.02 which was statistically non significant. Comparison of mean decrease between the experimental and control group were also found to be statistically non significant.

The mean decrease in mid arm circumference of both the groups were 0.17 ± 0.001 and 0.03 ± 0.08cm respectively. The decrease in mid arm circumference in both groups was found to be non significant.

The mean decrease in tricep skin fold thickness in experimental group was found to be 0.40 ± 0.01 mm and 0.13 ± 0.1mm respectively. The difference between the initial and final values were found to be statistically non significant in case of both groups.

On the whole results revealed that supplementation of herbomix for 60 days showed decrease in weight but statistically non significant. The results of other anthropometric indices also indicated the statistically non significant impact.

4.6 Lipid profile of selected subjects

4.6.1 Lipid profile of experimental group before and after supplementation

Hyperlipidemia is defined as increase in the lipid content in blood. As we move up the ladder of economic prosperity, diet associated chronic diseases begin to assume significance. The incidence of chronic diseases is increasing all over the world and is becoming a problem of significant importance. Abundant evidences are there to prove the link between hyperlipidemia and atherosclerosis. Combating heart disease is one of the challenging problems of medical science. Though there is advanced treatment, it is expensive and often beyond the reach of common man in

developing countries. Preparation of food in Indian kitchens by utilizing medicinal plants and fruits have been identified as hypolipidemic in Ayurveda due to presence of different phytochemicals, vitamins and minerals. It would be our advantage to prevent disease through several available approaches.

Therefore, an effort was made to prepare herbomix by utilising herbs such as amla, bottle gourd, safflower petals and tulsi leaves which has numerous health benefits.

The mean values of lipid profile in the serum of experimental group before (0 day) and after (30 and 60 days) supplementation are presented in Table 11 and illustrated in Figure2.

The mean values before supplementation for total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were 213.28 $\pm$ 4.93, 46.21 $\pm$ 14.93, 131.90 $\pm$ 15.94 an d176.03 $\pm$ 72.74 mg/dl respectively. At the end of 30 days after supplementation of herbomix the values obtained for correspondence parameters were 181.00 $\pm$ 9.67, 40.33 $\pm$ 9.75, 113.00 $\pm$ 13.57 and 136.60 $\pm$ 61.11 mg/dl respectively. Results indicated that there was decrease in total cholesterol, LDL cholesterol and triglyceride after supplementation of herbomix for 30 days.

The results of total cholesterol showed that the decrease in total cholesterol was statistically significant at 1 per cent level ($P<0.01$). However, the initial values and after 30 days of supplementation for triglyceride and LDL cholesterol the difference observed was not significant. Similarly the decrease in HDL cholesterol was recorded.

Mean values recorded after 60 days of supplementation for total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were 157.00 $\pm$ 13.59, 47.15 $\pm$ 47.15, 88.89 $\pm$ 18.65 and 105.10 $\pm$ 41.17. From results it is clear that there was marked reduction in total cholesterol, LDL cholesterol and triglyceride when compared to results obtained for 30 days. The

Table 11 Lipid profile of experimental group before and after supplementation (n=6)

Lipid profile	Zero days (before)		30 days		60 days (after)	
	Range	mean ± SD	Range	mean ± SD	Range	mean ± SD
Total cholesterol (mg/dl)	204 - 218	213.28 ± 4.93	168 – 195	181.00 ± 9.67	140 – 172	157.00 ± 13.59
HDL cholesterol (mg/dl)	26 – 70	46.21 ± 14.93	25 – 54	40.33 ± 9.75	40 – 56	47.15 ± 47.15
LDL – cholesterol (mg/dl)	104 – 144	131.90 ± 15.94	97 – 130	113.00 ± 13.57	140 – 172	88.89 ± 18.65
Triglyceride (mg/dl)	105 – 288	176.03 ± 72.74	82 – 217	136.60 ± 61.12	54 – 143	105.10 ± 41.17

't' values of lipid profile (students paired 't' test)

Day	Total cholesterol	HDL – cholesterol	LDL – C	Triglyceride
Zero Vs 30	7.281 **	0.814 ^{NS}	2.214 ^{NS}	1.014 ^{NS}
Zero Vs 60	9.533**	0.147 ^{NS}	4.292 **	2.089 ^{NS}
30 Vs 60	3.523*	1.457 ^{NS}	2.563*	1.057 ^{NS}

* Significant at 5% level ** Significant at 1% level NS Non significant

4.4.4 Adequacy in the intake of different nutrients per day by the control group

Per cent of adequacy in the intake of different nutrients per day by the control group is presented in Table 8.

The per cent adequacy values of fats and oils and Calcium for male respondent was 294.05 and 153.43. The respective values for corresponding nutrients for female respondent were 221.25 and 110.00 per cent. Adequacy recorded for fats and oils and calcium were more than RDA in both respondent. Per cent adequacy in supply of energy and protein was ranging from 75 – 80 per cent in both respondent groups. Per cent adequacy value for CHO intake in male respondent was observed to be nearer to 100 as 95.12 while in case of female respondent it was only 63.82.

In case of iron per cent adequacy was observed to be 54.17 and 37.96 in male and female respondent respectively. From results it was observed that diet taken by both respondent was deficit in supply of fiber as per cent adequacy is found to be less than 30.

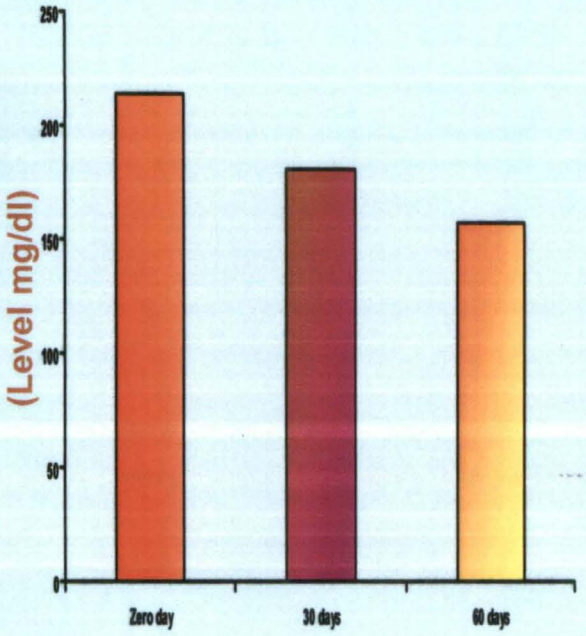
4.5 Anthropometric assessment.

The measurement of anthropometric indices are useful to assess the nutritional status of the individuals and thus helps to know health status of subjects.

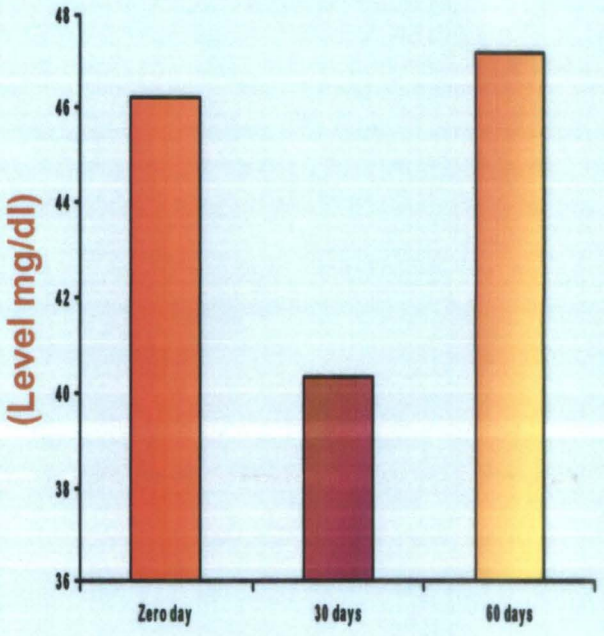
4.5.1 Anthropometric measurements of experimental group before and after supplementation.

The mean values of anthropometric measurements of the experimental group before (0 days) and after supplementation (30 and 60 days) are presented Table 9.

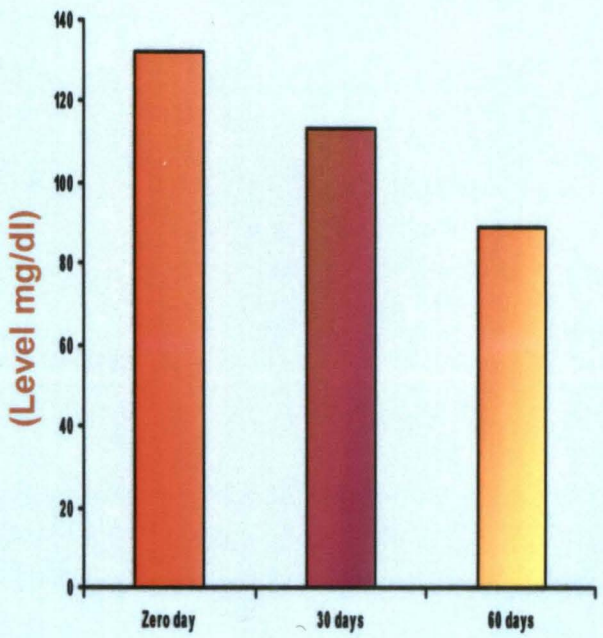
The mean value of body weight of experimental group before supplementation was 58.81 $\pm$ 6.97 kg and ranged between 50-68.40 kg. Whereas, after supplementation at 30 and 60 days it was 58.03 $\pm$ 6.91 kg and 57.58 $\pm$ 6.83kg respectively.



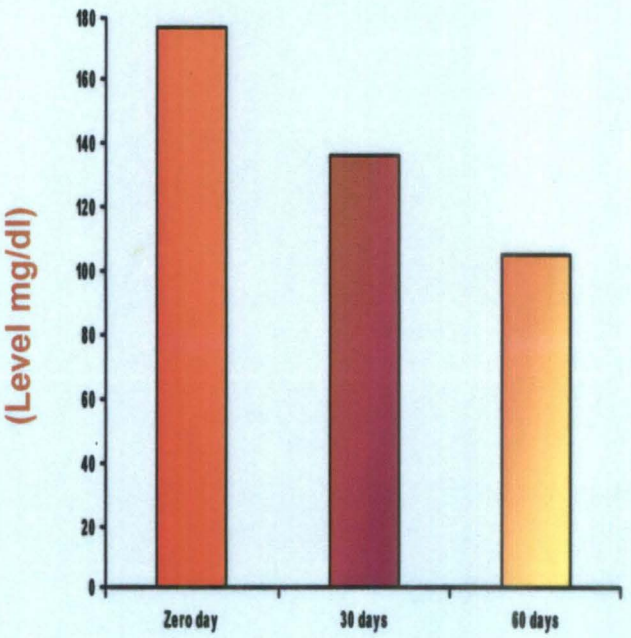
Total Cholesterol



HDL Cholesterol



LDL Cholesterol



Triglyceride

Fig 2 Lipid profile of experimental group before and after supplementation

difference observed was statistically significant for total cholesterol and LDL cholesterol ($P<0.01$) whereas increase in HDL cholesterol was observed from 40.33 ± 9.75 to 47.15 ± 47.15 but it was not significant.

The values obtained after supplementation of 60 days when compared with initial values (0 days) found that total cholesterol and LDL cholesterol decreased markedly. The decrease was statically significant at 1 per cent level ($P<0.01$). While the difference was observed in values of triglyceride for 0 to 60 days of supplementation but it was not significant. The values of HDL increased from 46.21 ± 14.93 (0 days) 47.15 ± 47.25 (60 days) but it was not significant.

On the whole it can be concluded from results of serum lipid profile of experimental group that the supplementation of herbomix for 60 days reduced total cholesterol, LDL cholesterol and triglyceride over initial values. Enhancement in level of HDL was observed which is a good cholesterol. The results of dietary intake of present study indicated that the consumption of fat in daily diet of subjects was more than RDA which may be one of the causative factor for the subjects being hyperlipidemic.

The marked reduction in total cholesterol, LDL cholesterol and triglyceoride of selected subjects after supplementation may be attributed to phytochemicals and other compounds present in herbomix prepared by utilising amla (gallic acid, tannins, flavonoids, pectin, Vitamin C, phyllambic compound) bottole gourd (Flucosterol, compestrol, pectin flavonoids, cucrbitacin sapoin. Flavon c glycosides). Safflower (gamma linolenic acid (n- 6) alpha linolenic acid (n – 3), glycosides, flavonoids) and tulsi (eugenol, urasolic acid, rosmarinic acid, flavonoids)

Shwetal *et al* (2006) investigated that the administration of *Ocimum sanctum* seed oil in rabbits decreased serum cholesterol, triglyceride and LDL cholesterol as compared to untreated group.

Iyer *et al* (2009) reported the supplementation of amla brought significant change in the lipid profile of the diabetic subjects, also total cholesterol decreased by 5.7 per cent. This could be due to the nutrient and phytochemical composition of amla.

Thamolwan (2009) reported that essential oil extract from *Ocimum sanctum* leaves in rats does not show significant effect on HDL cholesterol but it decreases significantly total cholesterol, LDL C and triglyceride. Plant foods are sources of phytochemicals that may have a useful role in the prevention of chronic diseases such as cancer, diabetes, cardio vascular disease, cateract and gall stone. Thus it can be concluded that supplementation of herbomix had exerted positive effect on serum lipid profile of subjects also it was observed that duration of supplementation as the period of supplementation increased the total cholesterol, LDL cholesterol and triglyceride decreased.

Hence the intake of herbomix can be recommended for the consumption of hyperlipidemic subjects.

4.6.2 Lipid profile of experimental and control group before and after supplementation

The changes in mean values of lipid profile of experimental and control group before and after supplementation of herbomix are presented in Table 12 and illustrated in Figure 3.

The mean decrease in the total cholesterol in both the groups were 56 $\pm$ 8.86 and 22 $\pm$ 0.56 mg/dl respectively. The decrease in total cholesterol in case of experimental group was found to be significant at one per cent level and in case of control group it was non significant. Besides the comparison of the mean decreases in the cholesterol between the experimental and control group showed statistical significance at five per cent level.

In case of supplementation of herbomix to experimental group the level of HDL cholesterol increased by 0.94 $\pm$ 32.32 mg/dl. The difference in

initial and final values was found to be non significant, on the other hand control group showed the decrease in HDL cholesterol by 14.99 ± 14.01 mg/dl but not significantly.

In case of LDL cholesterol it was observed that supplementation of herbomix in experimental group for 60 days declined the LDL cholesterol to the tune of 43.01 ± 72.95 mg/dl over initial values and difference was statically significant at one per cent level. While in control group the mean decrease was found to be 18.94 ± 27.79 mg/dl but it was statistically non significant.

The mean values of LDL cholesterol of experimental group obtained after 60 days of experimental period were statistically non significant when compared with control group (E vs C).

The results of study showed that supplementation of herbomix for 60 days exhibited the decrease in the triglyceride value over initial value among experimental group but the decrease was statistically non significant. Subsequently the same trend was noticed in control group. Even the comparison of mean decrease in the triglyceride between experimental and control group was non significant.

From findings it can be concluded that, supplementation of herbomix for 60 days in experimental group had exerted better impact of reducing total cholesterol, LDL cholesterol and triglycerides. On the other hand experimental group consuming herobomix for 60 days showed increase in HDL cholesterol.

It has been widely known that atherosclerosis is a serious complication produced by hyperlipidemia. It eventually causes coronary heart disease and in modern time the number of hyperlipidemic patients has been continuously increasing due to life style specially consumption of high fat diet. Numerous studies have indicated that diet regulation and drug therapy, to control blood cholesterol can subsequently reduce coronary heart disease, morbidity

Table 12 Lipid profile of experimental and control group before and after supplementation (n=12)

Parameters	Mean ± S.D.		Difference	't' values	
	Initial (0 days)	Final (60 days)		I VSF (0 Vs 60 days)	E Vs C (60 days)
Total cholesterol (Mg/dl)					
Experimental	213.28 ± 4.93	157.00 ± 13.59	56 ± 8.86	9.533**	6.33**
Control	235.00 ± 13.32	213.00 ± 13.88	22 ± 0.56	2.312 NS	
HDL Cholesterol (mg/dl)					
Experimental	46.21 ± 14.93	47.15 ± 47.15	0.94 ± 32.22	0.14 NS	0.65 NS
Control	57.70 ± 26.68	42.71 ± 12.67	14.99 ± 14.01	1.013 NS	
LDL – Cholesterol (mg/dl)					
Experimental	131.90 ± 15.94	88.89 ± 88.89	43.01 ± 72.95	4.292 **	1.24 NS
Control	134.49 ± 13.13	116.05 ± 40.92	18.94 ± 27.79	0.887 NS	
Triglyceride (mg/dl)					
Experimental	176.03 ± 72.74	105.10 ± 18.65	70.93 ± 54.09	2.089 NS	1.209 NS
Control	214.40 ± 2.67	156.00 ± 2.20	56.20 ± 0.47	0.854 NS	

I - Initial F - Final E - Experimental C - Control
* Significant at 5% level ** Significant at 1% level. NS non significant

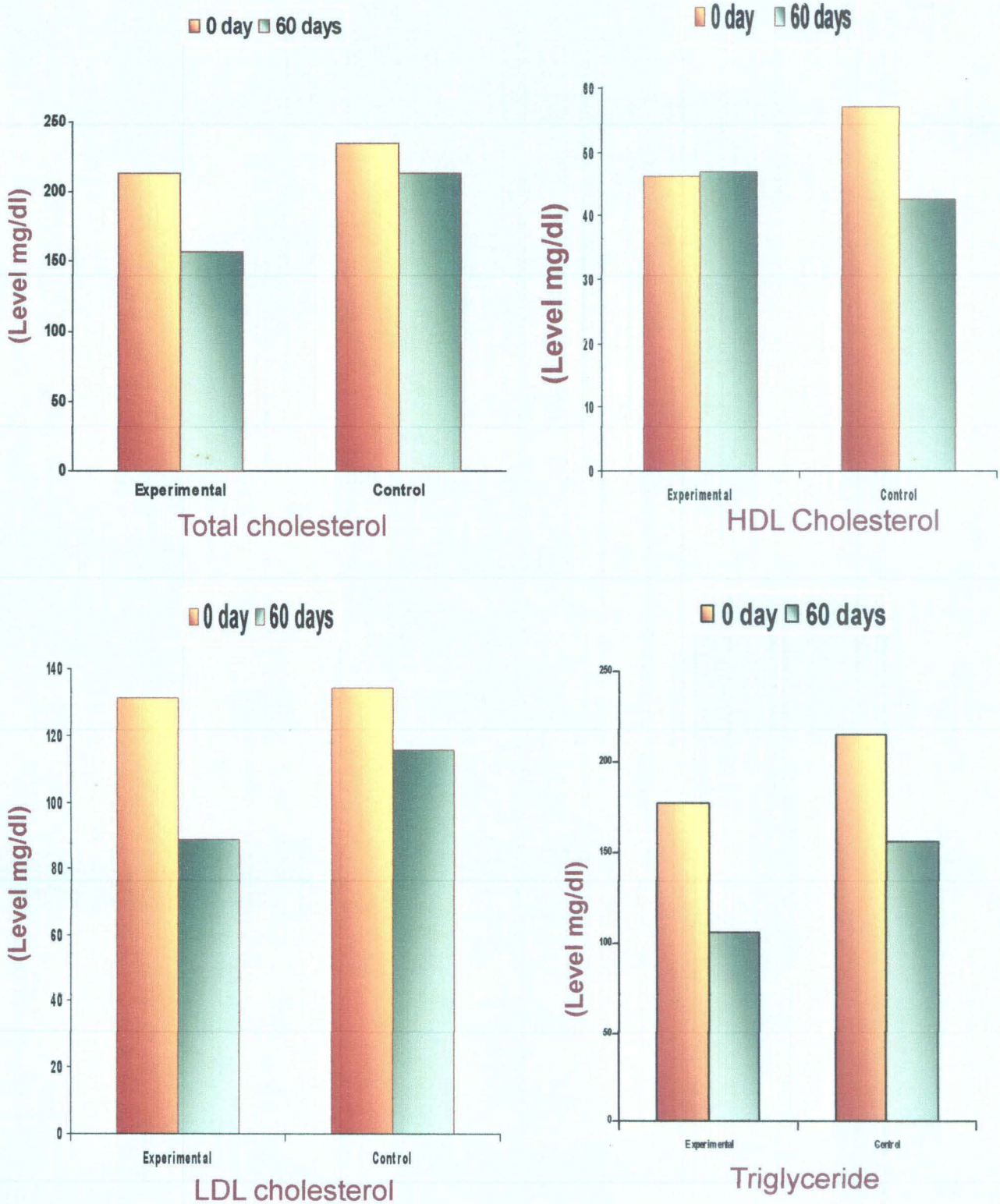


Fig 3 Lipid profile of experimental and control group before and after supplementation

and mortality (Kwiterovich, 1997). Synthetic lipid lowering drugs are useful in treating hyperlipidemia but there are number of adverse effects. Therefore herbomix was prepared by utilizing different herbs which have been shown to have a potential for lipid lowering action.

The activity of amla from medicinal point of view is attributed to high ascorbic acid contents and other compounds viz. tannins, alkaloids, phenolic compounds, amino acids. It is found that the amla functions at several different levels cardiovascular risk factors. The pure extracts of amla have now been shown to act in precise way to break the cycle of LDL oxidation, inflammation and plaque formation. Much of the excitement being generated about cardioprotection circles today is due to the fact that amla extracts not only reduce the oxidation of fat molecules in cell membranes and in blood. They actually reduce levels of dangerous fats while increasing levels of beneficial high density lipoprotein (HDL). Amla extracts inhibit LDL oxidation a first step in atherosclerosis more powerfully.

Indian medicinal plants are also important source of dietary fibre, vitamin, mineral antioxidans, oligosacchaxides, essential fatty acid (omega-3). Omega-3 fatty acids have been shown to lower serum cholesterol and triglyceride levels particularly in persons with hypertiglyceridemia by inhibiting the synthesis of very low density lipoprotein (VLDL), cholesterol and triglycerides. In the liver omega -3 and omega – 6 fatty acids are essential and they are not synthesized by the body and must be obtained through diet or supplementation. Linolenic acid is polyunsaturated fatty acids and can be converted to various fatty acids. The primary form of omega-3 fatty acids is linolenic acid. Nagraj (2002) reported that safflower petals contain gamma linolenic acid (n-6) and alpho linolenic acid (n-3) 2.7 and 16.8 per cent respectively.

Tulsi predominatly contain phenylopropanol compounds in which eugenol and methyl eugenol were main compounds. A number of biological

effects of eugenol and methyl eugenol have been reported including hypotensive, myrelaxant, antispasmodic and antioxidant effects (Klem *et al.* 2000). Moreover, eugenol has been shown to lower a high serum lipid profile in hyperlipidemic mice (German et al. 1998). Eugenol has been found to act as an a antioxidant and could inhibit LDL oxidation, thereby preventing atherosclerosis. (Rajlakshmi *et al.* 2000). It has been found that fresh leaves in the diet decreased the serum lipid profile in normal albino rats (Sarkar *et al.* 1994). Similarly supplementation of dried tulsi leaf powder in the diet suppressed the high lipid profile in diabetic rats (Suanarunsawat and Songsak 2005).

In conclusion results of present study demonstrate that developed herbomix possess hypolipidemic effect. Lipid lowering effect may be related to hypocholesteromic property of constituents existing in the herbs used for preparation of herbomix.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Plant foods are not only the source of nutrients but also rich source of bioactive phyto-chemicals or bio-nutrients. Studies carried out in the past have shown that these phyto chemicals have an important role in preventing chronic diseases and hypercholesterolemia. The medicinal value of plants lies in bioactive phytochemical constituents that produce definite physiological action on human body. Phytochemical is a natural bioactive compound found in plants. Such as vegetables, fruits, medicinal plants, flower, leaves and roots that work with nutrients and fibres to act as an defense system against disease. India is one of the most medicoculturaly divert countries in the world where medicinal plant sector is part of time honored tradition that is reputed even today. Medicinal plants are natures gift to human being to make disease free healthy life and are believed to be much safer. It plays a vital role to protect our health.

In spite of the presence of number of synthetic oral drugs in the market, researchers are now diverted their attention to different herbs and medicinal plants in order to find out new active principle with less side effect and better activity. Keeping in view the present study was planned with the main objective to explore effectiveness of herbomix supplementation on lipid profile along with regular routine diet, exercise and medicine.

The study was carried out in two phases. In first phase of study three variations of herbomix were developed utilizing amla (*Emblica officinalis*), bottle gourd (*Laqenaria siceraria*), safflower petals (*Carthamus tinetorius*), tulsi (*Ocimum sanctum*), honey and jaggery. Developed herbomix were

evaluated for organoleptic characteristics. Highly accepted herbomix was analysed for nutrient content.

In second phase of study human studies were conducted subjects having high lipid profile were selected following purposive sampling technique. The information regarding socio economic background, health status and food consumption pattern was collected by personal interview method using pretested questionnaire. The developed herbomix was supplemented to the selected subjects for 60 days and effect of supplementation on anthropometric measurements and lipid profile was determined, periodically at 0, 30 and 60 days. The observation recorded were tabulated statistically and summarized.

The results of organolepitc evaluation indicated that variation I recorded highest scores 4.3, 4.3, 4.2 4.3 and 4.4 for colour, texture, flavour, taste and overall acceptability respectively.

Nutrient content of highly acceptable herbomix variation I showed the moisture (27.41), protein (3.2), fat (0.4), total mineral (2.60), crude fiber (2.85) and CHO (63.54) g/100g. The values obtained for trace elements i.e. calcium, iron, copper, zinc and manganese (mg/100g) were 282.00, 10.2, 10.41, 18.16 and 7.3 respectively.

On the whole it can be said that developed herobmix provides more amount of nutrients like crude fibre, total minerals, calcium, iron, zinc, copper and low fat content which is beneficial for hyperlipidemic subjects.

The data on socio economic background and health status indicated that most of the subjects were belonging to middle income group, nuclear family, moderately educated and had history of disease more than five years and most of them were taking allopathic medicine.

The results of dietary and nutrient intake of different foods per day by the experimental and control group was inadequate in supply of cereals,

fruits, green leafy vegetables and sugar and jaggary. On the other hand per cent adequacy of fats and oils was found to be more than RDA.

Percent adequacy of different nutrients per day by the experimental and control group showed that consumption of fat and calcium was more than RDA whereas, energy, protein, carbohydrate and iron was found to be less. Further diets was grossly deficient in supply of fibre in both groups.

The anthropometric measurements of experimental and control group before (0days) and after (60 days) supplementation of herbomix were recorded. The results showed that the corresponding values of the weight ranged between 58.81 to 57.58 kg and 61.72 to 61.45 kg for body mass index 24.81 to 24.29 and 26.57 to 26.72 for mid arm circumference 28.10 to 27.93 cm and 25.37 to 25.40 and for tricepskin fold thickness 30.05 to 29.65 mm and 29.95 to 29.82 mm respectively.

On the whole supplementation of herbomix for 60 days showed non significant effect on body weight, body mass index, mid arm circumference and tricep skin fold thickness, but slight decrease in body weight was observed, thus long term supplementation may prove the efficiency of herbomix.

The initial mean values for total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride of experimental group was 213.28 $\pm$ 4.93, 46.21 $\pm$ 14.93, 131.90 $\pm$ 15.94 and 176.03 $\pm$ 72.74 mg/dl of serum respectively. The values obtained after supplementation of herobmix at the end of 60 days of the study period for the corresponding parameters were 157.00 $\pm$ 13.59, 47.15 $\pm$ 47.15, 88.89 $\pm$ 18.65, 105.10 $\pm$ 41.17 mg/dl respectively.

On the whole it was concluded that the supplementation of herbomix to experimental group for 60 days reduced significantly total cholesterol and LDL cholesterol over initial value, decreased in triglyceride was also observed but not significantly. Enhancement in the beneficial HDL cholesterol was observed which is considered to be good cholesterol.

The initial mean values for total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride of control group were 235.00 ± 13.32, 57.70 ± 26.68, 134.49 ± 13.13, 214.40 ± 2.61 mg/dl of serum respectively. The values obtained at 60 days for control group were 213 ± 13.88, 42.71 ± 12.67, 116.05 ± 40.92 and 156.00 ± 2.20 respectively.

In a nutshell organoleptic characteristics of variation I was found to be most acceptable and contained high amount of crude fibre, total minerals, calcium, zinc and copper.

The results of dietary intake of selected subjects showed that diet was in adequate in intake of cereals, fruits, green leafy vegetable's sugar and jaggary where as intake of fats and oils was found to be more than RDA. In case of nutrient intake it was found that the intake of energy, protein, CHO, iron and fibre was less but it was vice versa for fat and calcium.

The findings of study revealed that the supplementation of herbomix did not show significant effect on body weight, body mass index, mid arm circumference and skinfold thickness of selected subjects.

The results of lipid profile showed that the supplementation of herbomix for a period of 60 days decreased significantly total cholesterol and LDL cholesterol. However non significant decreased in triglycerides was also noticed. Further more enhancement in the HDL cholesterol was observed. In conclusion it can be said that supplementation of herbomix exerted positive effete on serum lipid profile of selected subjects.

Plant essential oil and extracts have been used for many thousand years, in food preservation, pharmaceutical medicine and as natural therapies. Therefore, it is necessary to exploit its maximum potential infield of medicinal and pharmaceutical sciences for fruitful application. The present study clearly elicited that herbomix prepared by utilizing herbs like amla, bottle gourd, safflower and tulsi are efficient as a lipid lowering agent may be due to presence of phytochemicals and antoioxidants.

Herbal medicine is increasingly gaining greater acceptance from public and medical profession due to greater advances in understanding mechanisms by which herbs positively influence health and quality of life and also easily affordable by the community. An impressive array of health promoting, vitality enhancing properties of herbomix in addition to many more therapeutic actions like account for much of the exceptionally broad range of medicinal uses and can provide some helps in pratternising indigenous drugs in treatment of hyperlidemia. Supplementation of herbomix for a longer period had exerted a positive impact on the studied parameters of selected subjects.

The natural compounds present in herbomix are safflower yellow and red pigment, glycoside, benzylglycoside, gamma linolenic, acid (n-6), alphalinolenic acid (n-3), eugenol ursolic acid, apigenin, alkaloids, flavonois, gallic acid, ellagic acid, vitamin C, crude fibre and many more.

Tulsi protects and reduces stress, enhances stamina and hindrance, increases body's efficient use of oxygen, boosts the immune system reduces inflammation, protects against radiation, damage, support heart, liver and lungs, has antibiotic, antiviral and antifungal properties, enhances the efficacy of many other therapeutic treatment and provides a rich supply of antioxidants and other nutrients.

Amla has effectiveness as an antioxidant, antidiabetic and antihyperlipidemic. In addition to amla has documented effectiveness at reducing serum cholesterol. The extract appears to be cardio-protective, offering protection against atherosclerotic plaque formation. Studies with chronic stress induced changes in various tissues, including brain, kidney, liver and heart postulate that amla has ability to reserved damage and protect tissue may be due to amlas free radical scavenging activity.

Besides herbs have antioxidant, hypolipidemic, hypoglylcemic, hypotensive, antimicrobial, anti inflammatory, anticarcinogenci,

antidiarrheal, antistress, antiulcer, antifertility, antidiabetic, antiasthamatic, carminative, diuretic and laxative properties.

Nevertheless, the results of present study deserves attention as the decrease in total cholesterol is more significant and may prompt other research group to explore amla, bottle gourd safflower and tulsi as a safe drug to treat hyperlipidemia through well controlled randomized trial group.

On the whole it can be concluded that herbomix had significant hypocholesterolemic activity. In addition it did not show any toxic effect and found to improve the health status of selected subjects. Therefore, this developed herbomix can be selected for further research work to isolate the active phyto-chemicals and antioxidant activity. Further the impact of supplementation of herbomix for a longer period can be studied.

LITERATURE CITED

LITERATURE CITED

A.O.A.C. (1975) Official methods of analysis Association of Official analytical chemist. 14th Edn. Washington, DC.

Agarwal P. (1996). Medicinal herb tulsi. Int. J. Clin. pharmacol Ther, 34 (9) : 406-409.

Anabarasu, K. and Vijayalakshmi (2007). Improved shelf life of protein rich tofu using *Ocimum sanctum* (tulsi) extracts to benefit. Indian Rural Population. Innovations in food science and Technology pp 35

Anila L. and N.R. Vijayalakshmi (2002) Flavonoids from *Emblica officinalis* and *Magnifera indica* effectiveness for dyslipidemia. J. Ethnopharmacol. 79 (1) : 81-7.

Anita Kochar, Neha Sharma and Rajbir Sachdeva (2008) Effect of supplementation of Tulsi and neem leaves on blood glucose and serum lipid profile of non insulin dependant diabetics. Ind.J.Nutr.Dietet.45:11-16.

Ansarullah, Jadeja, R.N., Thounaojam, M.C., Patel V, Devkar R.V. and Ramchandran A.V. (2009) Antihyperlipidemic potential of polyherbal preparation on trition WR 1339 (Tyloxopal) induced hyper lipidemia : a comparison with lovasatain : I.T.G.F. 3 (2) : 119-124.

Asadul Haque S.K., Sen, D., Bagchi U.B., Chakrabarty M.M. and Sunit Mukherjee (2006). Evaluation of total antioxidant capacity of some vegetables. Spices and tea. J. Food Sci. Technol. 43 (5) : 467-469.

Bajaj Seema and Satwadhar, P.N. (2006). Studies on preparation and standardization of herbal drink from Amla. Innovations in food science and technology, pp 83

Bhattacharya A. Chatterjee, A., Ghosal S and Bhattacharya, S.K (1999) Antioxidant activity of active tanoid principles of *Emblica officinalis* (amla). Indian J. Exp. Biol., 37 : 676 -680.

Chang, S.C. Lee, M.S., Li Ch and Chen MI (1995). Dietary fiber content and composition of vegetable in Taiwan area. Asian Pacific J. Clin. Nutrn. 4 : 204-210.

Chopra B. N. and Chopra I. C. (1992). Glossary of Indian Medicinal Plants. Publication and Information Directorate, CSIR, Council of Scientific and Industrial Research, New Delhi, India.

Deodhar, S.K. (2001) Evaluation of medicinal value of safflower petals in hypertensive and hyperlipidemic subjects. M.Sc. thesis submitted to Dept. of Foods and Nutrition, College of Home Science, MAU, Parbhani

Deore, P.M., Kotecha, P.M. and Pawar, V.D. (2008) Studies on processing bottle gourd into juice and powder. Indian food packer 116-120.

Deshpande, D. (2008) Handbook of herbal remedies.pp:

Deshpande, J.R., Mishra, M.R., Meghre V.S, Wadodkar S.G. and Dorle A.K. (2007). Free radical scavenging activity of *Lagenaria siceraria* (mol) standl fruit. Natural product Radiance 6(2) : 127-130.

Dhananjay L., Samudralawar and Amar, N. (1996) Minor and trace elemental determination in the Indian Herbal and other medicinal preparations. Biological Trace Elements Research, Vol 54, pp 113-115.

Edwin Jarald, Omprakash Bangar, Sheeja Edwin, Showkat Ahmad, Shamsuddin Jamalludin (2009). Antidiabetic activity of few Indian medicinal plants vs their combination in Alloxan induced diabetic rats. J. Pharmacy Research 2 (11) : 1760-1763.

Erasto, P. and Z.H. Mbwambo (2009). Antioxidant activity and HPTLC profile of *Lagenaria siceraria* fruits. Tanzania J. Health Research 11(2) : 79-83.

Garg, Vivek, Barwal Y.S. and Sanjay Sarera (2008) Preparation and evaluation of vitamin C enriched fruit drink. J. food Sci. Technol. 45 (6) : 524-526.

German C, Leticia G, Adrians, Fermandol, Maria S., Elizadath M., Francisco D., Joaquint (1998) Hypolipidemic activity of dimethoxy uncojugated propenyl side chain analogs of α- asarone in mice. drug. Dev. Res. 43:105 – 108

Ghule B.V., Ghante, M.H. Saoji, A.N. and Yeole, P.G. (2006). Hypolipidemic and antihyperlipidemic effect of *Lagenaria Siceraria* (mol) fruit extract. Ind. J. Exp. Biol. 44 : 905-909.

Ghule B.V., Ghante, M.H. Saoji, A.N. and Yeole, P.G. (2006). Diuretic activity of *Lagenaria Siceraria* fruit extract in rats. Ind.J. Pharm.Sci.69(6),817-819.

Gopalan, C., Rama Sastri B.V. and Balasubhramanian S.C. (2004). Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad 500 007 India.

Gopalan, G., Rama Sastri, B. and Balsubramaniams, (2000). Revised and updated by Narasingarao B., Deosthale Y. and Port. K., National Institute of Nutritional Indian Council of Medical Research

Gupta S.K.and Prakash J.(2002). Validation of traditional claim of Tulsi, Ocimum sanctum linn as a medicinal plant. Ind.J.exp. Bio.40(7) 765-773

Halim Eshrat M. and A.K. Mukhopadhyay (2006) Effect of *Ocimum sanctum* (Tulsi) and vitamin E on biochemical parameters and retinopathy in streptozotocin induced diabetic rats. Ind. J. clinical Biochem. 21 (2) 181-188.

Hassanpour Fard, Bodhankar, S.L. and Dikshit Madhumira. (2008). Cardio protective activity of fruit of *Lagenaria sicerana* (Molina) Stanlay on Doxorubicin induced cardiotoxicity in rats. Int. J. Pharmacol 4 (6) : 466-471.

Hubert H. B., et al. (1983). Obesity as in independent risk factor for cardiovascular disease : a 26 year follow – up of participants in the framingham Heart Study, Circulation 67 : 968.

Ingole, A.J. (2007). Evaluation of therapeutic value of chutney prepared with incorporation of safflower petals powder in hypertensive subjects. M.Sc. thesis submitted to Dept. of Foods and Nutrition, College of Home Science, MAU Parbhani

Iyer, U. Joshi A. and Dhruvs (2009). Impact of Amla (*Emblica officinalis*) supplementation on the glycemic and lipidemic status of type 2 diabetic subjects. J. Herbal medicine and Toxicology 3 (2) : 15-21.

Jacob, A., Pandey, M., Kapoor S. and Saroja R. (1988). Effect of the Indian gooseberry (amla) on serum cholesterol levels in men aged 35-55 years. Eur. J. Clin. Nut. 42 (11) : 939-44.

Jain S.K. and D.S. Khurdiya (2004). Vitamin C enrichment of fruit juice based ready to serve beverage through blending of Indian gooseberry (*Emblica officinalis* Gaertn) Junice. Plant foods Hum. Nutr.,59 (2) : 63-6.

Javanmardi J., Stushnoff C., Locke E., Vivanco JM (2003). Antioxidant activity and total phenolic content of Indian Ocimum accession. Food chem.. 83:547-550.

Juntachote T. and Berghofer E. (2005). Antioxidative properties and stability of ethanolic extracts of holy basil and galangal Food Chem. 92 : 193 – 202.

K.H. Khan (2009). Roles of *Emblica officinalis* in medicine. A review Botany Research International 2 (4) : 218-228.

Kafka S. R., Karney T. E., Knowles P. D. and Miller M. D. (2006). Safflower production in California. Dept. of Agronomy and Range Science University of California, davis Web Master Agric. Undavil, Edu.,:1– 4.

Karla CL (1988). The chemistry and technology of amla (*Phyllanthus emblica*) a resume. Indian Food Packer 42 (4) : 67-80.

Kelm M. A., Nair M. G., Strasberg G. M., De Witt dl. (2000). Antioxidant an cycloxygenase inhibitory phenolic compounds from Ocimum sanctum phytomedicine 7 : 7 – 13.

Khan, M.R.I., Islam, M.A., Hossain, M.S., Asadujjaman, M., Wahed, M.I.I., Rahman, B.M., Anisuzzaman, A.S.M., Shaheen, S.M. and Maruf Ahmed (2010). Antidiabetic effects of the different fractions of ethanolic extracts of *Ocimum sanctum* in normal and Alloxan induced diabetic rat, J. Sci. Res. 2 (1) : 158-168.

Khan, T.N. (2005). Evaluation of safflower petals for therapeutic value in selected disease. Ph.D. thesis submitted to Dept. of Foods and Nutrition, College of Home Science, MAU Parbhani

Krishnaiah D., Sarbatly, R. and Bono A. (2007) Phytochemical antioxidants for health and medicine A move towards nature. Biotechnol. Mol. Biol. Rev. 1 (4) : 97 – 104.

Kwiterovich Po, (1997). The effect of dietary fat, antioidant and pro oxidants on blood lipids, lipoprotein and atherosclerosis. J. Am. Diet. Assoc. 97 (suppl) : 531 – 541.

Li Dajue, Yunzhou, H and Wan L. (1989). Safflower research in China. In proceeding of IInd International safflower conference, Hyderabad India 9-13.

Li U (1989). The development and exploitation of safflower tea. Institute of Botany Chinese Academy of Sciences, Beijing 100093, China

Li. Dajue and Mundel, H.S. (1998). Promoting the conservation and use of under utilised and neglected crops 7 : safflower *Carthamus tinctarus* L., published by IPGRI.

Maime (2004). Maimes Report on Holy Basis. *Ocimum sanctum* Tulsi. www.holy.basil.com version 1, November

Mamila V. (2009). Impact of herbal composite supplementation on lipid profile of selected subjects M.Sc. thesis submitted to Dept. of Foods and Nutrition, College of Home Science, MAU Parbhani

Manny, NS, Shadaksharaswamy M (1997). Foods facts and principles. New Age International (p) Ltd. New Delhi p 190.

Mathur, Arti Sharma, V.P. Dixit and Mira Varma (1996). Hypolipidemic effect of fruit of *Emblica* officinalis in cholesterol fed rabbits. J. Ethnopharmocology. 50 (2) 61-68.

Matsuba shigeru,Yamada Nariko, yamazaki Takuya, Tabuchi Mihoko and Onodera Junichi(2003)Antioxidative activity of safflower components in rats. J. of Japanese society of Nutrition and food science,56(6)"365-369.

Merai, M., Boghra, V.R. and Sharma, R.S. (2003). Extraction of antioxygenic principles from tulsi leaves and their effects on oxidative stability of ghee. J. Food Sci. Technol. Vol. 40 (1) : 52-57.

Mohale, D.S., Dewani, A.P., Saoji, A.N. and Khadse C.D. (2008). Antihyperlipidemic activity of isolated constituents from the fruits of *Lagenaria siceraria* in albino rats Int. J. Green pharmacy. 104-107.

Nagraj G. (1993). Seed composition of fatty acid profile of some Indian safflower cultivars. Proc. III int. safflower conference. Beijing China, Jun 14-18 246-249

Naresh Khanna, Deepika Arora, Sumita Halder, Ashish K. Mehta, Gobind R. Garg, Suman B. Sharma and Prabha Mahajan (2010) Comparative effect of *Ocimum sanctum*, commiphora mukul, folic acid and ramipril on lipid peroxidation in experimentally induced hyperlipidemia. Ind. J. Exp. Bio 48 : 299-305.

NIN (1983). Nitrogen estimation by micro-kjedhel method NIN manual pp4

Ntezrubanza, L., Scheffer, J.J. and Looman, A. (1985) Composition of the essential oil of *Ocimum sanctum* grown in Rwanda. Pharm Weekbl. Sci., 7 (6) : 273-276.

Ojiako, O.A. and Igwe C.U. (2007). Nutritional and anti-nutritional compositions of cleome rutidosperma, *Lagenaria siceraria* and cucurbita maxima seeds from Nigeria. J. Med. Food 10 (4) : 735-8.

Panse, V.G. and Sukhatme, P.V. (1985). Statistical methods for agricultural works. ICAR publications New Delhi 58-60, 97-110.

Potwale (2008) Phyto constituents and therapeutic potential of *Phyllanthus Emblica*. A Review of Pharmacology online, 2 : 236 : 255.

Premi B.R., Vijay Sethi and Geeta Bisaria (2002). Preparation of instant oilless pickle from aonia (*Emblica officinalis* Gaertn). Indian food Packer, 72-74.

Pruthi J. S. (1998). Spices and condiments (India the land and the People) 5th edn. National Book Trust,, India.

Rahaman A.S. (2003) Bottle gourd (*Legenaria siceraria*) a vegetable for good health. National product Radiance, 2003 2(5), 249-256.

Rai V., Iyer U., and Mani U.V.,(1997). Effect of Tulsi leaf powder on blood sugar levels, serum lipids and tissue lipids in diabetic rats . Plant foods for Human Nutrition.50:9-16.

Rajalakshmi K., Gurumurthi P., Devaraj S. N., (2000). Effect of eugenol and tincture of cratagegus (TCR) on in vivo oxidation of LDL + VLDL isolated from plasma of non insulin dependant diabetic patients. Indian J. Exp. Biol., 38 : 509 – 511.

Rane A. C., Gangwal a., Parmar S. K., Gupta G. L. and Sheth N. R. (2008) Immunomodulatory effects of Laqenaria Siceraria fruits in rats. Pharmacoghosy Magazine, 4 (16) : S234 – S238.

Rangana, S. (1979). Manual of analysis of fruits and vegetables products. 2nd Edn. pp 281

Revanwar M. (1996)., Studies on extraction and uses of natural pigments from safflower florets. M.Tech. Thesis submitted to College of Food Science and Technology MAU Parbhani

Rumeza Hanif, Zafer Iqbal, Mudassar Iqbal, Shaheena Hanif and Masooma Rasheed (2006). Use of vegetables as nutritional food role in human health. J. of Agri. and Bio. Sci. Vol. 1 (1) : 18 – 22.

Sarkar, A.,M Lavania, SC, Pandey DN, Pant, MC (1994). Changes in the blood lipid profile after administration of *Ocimum sanctum* (Tulsi) leaves in the normal albino rabbits. Ind. J. Physiol. Pharmocol. 38 : 311-312.

Sawant A., Sutar, P. and Iranna, U. (2006). Preparation of new chawanprash. 18th Indian convection of food science and technology. pp 56.

Sawate A.R., Bhokre, C.K., Kshirsagar, R.B. and Gadge K.S. (2008) Evaluation of RTS beverage from bottle gourd. Ind. J. Nutr. Dietet. 45 : 371.

Shirwaikar, A and Sreeniwasan K.K. (1996). Chemical investigation and antihepatotoxic activity of the fruits of *Lagenaria siceraria*. Ind. J. Pharm. Sci 58 (5) : 197-205.

Shriniwas C.V.S., Praveena B. and Nagraj G. (1999). Safflower petals : A source or gama linolenic acid. Plant Foods and Human Nutrition, 54 : 89-92.

Shweta Gupta, Pramod K. Mediratta, Surender Singh, K.K. Sharma and Rimi Shukla (2006). Antidiabetic, antihypercholesterlaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. Ind. J. Experimental Biology 44 : 300-304.

Solunkhe, D.K., Chavan, J.K., Adusle R.N. and Kadam S.S.(1992). Safflower world oilseed chemistry technology and utilisation : 326-363.

Srinivas C.V.S., Praveena, B. and Nagaraj G. (1999). Safflower petals : A source of gama linolenic acid. Plant foods for human nutrition. 54 (1):89-92.

Stark RET (1987). Body mass index. The Bariatrician 4 : 20, winter.

Sunitha, T., Manovama, R., Rukmini, C. (1997). Lipid profile of rats fed blends of rice bran oil in combination with safflower and safflower oil plan foods far human nutrition, 51 (3) : 219-230, 31 ref.

Suanarunsawat Watcharaporn Devakul Na Ayutthaya, Thanapat Songsak, Jittraporn Rattanamahaphoon (2009). Anti lipidemic actions of essential oil extracted from *Ocimum sanctum* L. leaves in rats fed with high cholesterol diet. J. Appl. Biomed. 7 : 45-53.

Tiwari, S. Chopra, C.S. and Pratibha Singh (2006). Preparation of products from sulphite treated stored aonla pulp (*Emblica officinalis* Gaertn). Beverage and food world. pp 41-42.

Vani, S.R., Cheng, S.F. and Chuah, C.H. (2009). Comparative study of volatile compounds from *Genus ocimum*. American Journal of applied Sci. 6 (3) : 523-528.

Vanitha Reddy, Asha Urooj and Anila Kumar (2005) Evaluation of antioxidant activity of some plants extracts and their application in biscuits. Food chemistry, 90 (1-2) pp 317-321.

Veena, K., P. Shanthi and P.Sachdanandam (2006). The biochemical alternation following administration of kalpaamruthaa and semecarpus anacardium in mammary carcinoma. Chem. Biol. Interact, 15; 161 (1) 69-78.

Vijayanand, P., Kulkarni S.G. and Pratibha G.V. (2006). Processing of Gooseberry into Gooseberry juice concentrate : A potential source of nutrients. Innovations in food science and technology pp 71

W. Guirong *et al.* (1995). The health care effect of safflower oil and the safflower resources in Xinjiang. Information of preventive medical 1 (2) : 299-300.

Wang Zhaomu and Du lijie (2001). Current situation and prospects of safflower products development in china In : proceedings of Vth International safflower conference, Williston, N.D. USA 315-319.

Wang Zhaomu and Fan Lin (1989). Safflower in Xinjiang. In proceeding of II international safflower conference Hyderabad India 75-77.

Wu, S Fu Jianxiang and Zhang Rui (1993). The research and production of carthamin, In : Proceeding of III international safflower conference, Beijing, China 14-18 June, 1993, 881-889.

Xiaoli Liu, Mouming Zhao, Jinushul Wang, Baoyong and Yueming Jiang (2007). Antioxidant activity of methanolic extract of emblica fruit from six region in China. J. Food composition and analysis 21 (3) : 219-228.

Yang Li Zhang (1993). The study of new and medicinal oil safflower, hospital of traditional Chinese medicine. Jimosare country, Xinjiang 831700, china.

Yang Li Zhang (1993). The study of new and medicinal oil safflower, hospital of traditional Chinese medicine. Jimosare country, Xinjiang 831700, china.

ANNEXURE

ANNEXURE- I

Proforma for the Organoleptic Evaluation of the Products

DEPARTMENTOF FOOD AND NUTRITION
COLLEGEOF HOME SCIENCE
MARTHWADA AGRICULTURAL UNIVERSITY, PARBHANI

Sensory evaluation of the product :

Name of Judge :

Designation :

Variations	Colour	Texture	Test	Flavour	Overall acceptability
I					
II					
III					
IV					

Scores

Excellent - 5
Very good - 4
Good - 3
Fair - 2
Poor - 1

Signature

ANNEXURE- II

Survey Schedule to assess the Socioeconomic and Health status of the selected subjects

A) General Information :

- Name of the City :
- District :
- Area :
- Name :
- Age :
- Sex :
- Address :
- Type of family : Nuclear / Joint
- Literacy level :

  Primary

  Secondary

  High school

  College
- Occupation :
- Monthly income :

  Rs. 5,000 – 10,000 [Group - I]

  Rs. <10,000 – 15,000 [Group - II]

  <Rs. 15,000 [Group - III]
- Food habits : Vegetarian / Non vegetarian
- Meal Pattern : Two / Three / Four

B) Anthropometric measurements :

- ✓ Height (cm.)
- ✓ Body Weight (kg.)
- ✓ BMI
- ✓ Triceps skin fold thickness (mm)
- ✓ Mid arm circumference (cm.)

C) Information regarding health status

- ✓ Are you suffering from any disease : Yes / No
- ✓ If yes, name of the disease :
- ✓ Duration of the disease :
- ✓ Are you taking any medicines regularly : Yes / No
- ✓ Types of medicines : Allopathy / Homopathy / Ayurvedic

D) Recall Method of food Consumption pattern for consecutive day

Meal Pattern	Time	Name of the Item	Food ingredients consumed by the subjects	Amount in raw quantity (gm)
Breakfast				
Mid Morning				
Lunch				
Snacks				
Dinner				
Bed Time				

E) Nutrition consumed in a day

Food stuffs	Ingredients used in preparation (Raw Foods)	Amount (gm)	Nutrient intake						
			Energy (kcal)	Protein (gm)	Fat (gm)	CHO (gm)	Fibre (gm)	Calcium (mg)	Iron (mg)

ANNEXURE- III

1. Determination of moisture content

Moisture content of the highly accepted herbal composite variation was determined by oven drying method of A.O.A.C. (1975)

Procedure

Three samples of highly accepted herbal composite were accurately weighted in an amount of 5.0 g each in weighing bottle (previously heated at 90° C to 100° C and cooled in desicator). The bottles were loosely covered with lids which contain sample transferred to oven for 3 hours at 105° C. After 3 hours bottles were removed from oven, allowed to cool in desicator and weighed accurately. Then again bottles were returned to oven 1 hour and weighed. This procedure was repeated until the constant weight was observed. Moisture content of sample was calculated by the formula.

$$\text{Moisture Contents of sample (\%)} = \frac{W_1 - W_2}{\text{Wt. of Sample (gm)}} \times 100$$

Where,

W_1 - Initial weight of bottle with sample before drying.

W_2 - Final weight of bottle with sample after drying.

1 Determination of total protein content of selected samples

Total protein content of the samples were estimated by determining total nitrogen content using standard macro-kheldhal method (N.I.N., 1983). Total protein was calculated by multiplying the estimated total nitrogen content with factors 6.25.

1.1 Preparation of regents

2.1.1 Catalyst mixture

It was prepared by grinding together 98 parts of potassium sulphate (K_2SO_4) and parts of copper sulphate ($CuSO_4$).

2.1.2 40 per cent sodium hydroxide

An amount of 40 g of sodium hydroxide pellets were dissolved in distilled water and diluted up to 100ml.

2.1.3 Methyl red indicator

2.1.4 2 per cent boric acid solution

A weighed amount of 2 gm of boric acid was dissolved in distilled water and volume was made 100ml.

2.1.4 0.1 N sulphuric acid

A measured quantity of 27.8 ml of concentrated suiphuric acid was dissolved in distilled water and the volume was made up to 100 ml. This solution given 1 n sulphuric acid solution was diluted up to 1000ml with distilled water.

Procedure

One gram of defatted powdered sample of highly accepted herbomix composite were weighed on a butter paper, in triplicate and placed in 500 ml kheldhal flask. An amount of 5.0 gm of catalyst mixture, 20ml of concentrated sulphuric acid and 203 glass bids were added each flask. Similarly blank was also prepared using other reagents except sample. The contents in the flask were digested by heating for about 8 hours until the digested material was clear. The contends were allowed to cool and diluted by rinsing down the neck of the flask with distilled water. The contents were then transferred to a 100ml. volumetric flask and the volume was made up to mark with distilled water.

10 ml of boric acid solution was delivered in to a 100 ml conical flask and two drops of methyl red indicator were added and mixed well. The flask was then placed under the condenser extending below the surface of boric acid solution; 5 ml of digested sample was delivered into the distillation apparatus. Than 10 ml of 40 per cent NAOH was added and the funnel was added and funnel was washed with 2 to 3 ml of distilled water. Steam distillation was carried out and it was continued for 15 min. until about 40 ml of distilled collected in boric acid solution. The tip of condenser was washed with distilled water and the flask was removed.

The ammonia collected in boric acid was titrated against the standard 0.1 N sulphuric acid solution. The end point of the titration was noted when 0.1 N sulphuric acid production a light pink colour. Then the volume of 0.1 N sulphuric acid required to neutralize the collected sample was noted.

Total protein content of sample was calculated by formula

Protein (%) = Nitrogen (%) X 6.25

Where,

$$\text{Nitrogen (\%)} = \frac{(\text{Titre value of sample}) - (\text{Titre value of blank}) \times \text{Normality of sulphuric acid} \times 14 \times 100 \times \text{dilution factor}}{\text{Wt. of sample (mg.)}}$$

Determination of total fat content

The fat content of selected sample was estimated by the soxhlet method of A.O.A.C. (1975)

Procedure

Three soxhlet flask of 250 ml capacity were cleaned and dried in an oven. Then three samples of highly accepted herbomix in an amount of 2.0 g were accurately weighed sample was placed in thimbles and plugged with fat free cotton. Then the thimbles with weighed sample were placed in the siphon portion of soxhlet apparatus. The volume of 160 ml analytical grade petroleum either and diethyl ether mixture (1:1) was placed in each round bottom flask of the soxhlet apparatus and it was connected to the soxhlet siphon and condenser. The condenser was plugged with a moistened cotton. It was refluxed for 5-7 times at 600 C. Then ether was distilled off and flask were placed on hot plate for 3 hours at 105^0 C for drying, cooled in a desecrator and weighted. Fat content of sample was calculated by using the formula.

$$\text{Fat content (\%)} = \frac{W_2 - W_1}{X} \times 100$$

Where,

W_2 - Weight of round bottom flask with fat

W_1 - Weight of empty round bottom flask

X - Weight of sample

3. Determination of crude fibre content

Crude fibre content of selected samples was determined by the method of A.O.A.C. (1975).

3.2. Preparation of reagent

3.2.1. 0.225 N sulphuric acid solution

A measured quality of 1.25 ml of concentrated sulphuric acid was dissolved n glass distilled water and volume was made up to 100ml.

3.2.2.1. 0.313 N sodium hydroxide solution

A weighed amount of 1.25 gm of sodium hydroxide was dissolved in glass distilled water and the volume was made up to 100ml.

Procedure

Exactly 2.0 g of moisture and fat free sample was weighed in triplicate in a 500ml of beaker and mixture was allowed to boil for 30 min keeping the volume constant by addition of water at frequent intervals, glass rod was used to stir the solution which helped for smooth boiling. Then the mixture was filtered through a muslin cloth and residue was washed with hot water to make it free from acid. The material was then transferred to the same beaker carefully; 200ml of boiling 0.313 N sodium hydroxide was added and boiling for 30 min keeping the volume constant by using distilled water. The mixture was again filtered through a muslin cloth and residue was washed with hot water till it was made free from alkali and some alcohol. Then residue was transferred to crucible which was dried in an oven overnight at 80° C and weighed accurately (W_1). The crucible was heated in muffle furnace at 600° C for 2-3 hours, cooled in a desiccators and weighed again accurately (W_2).

The difference between the two weights (W_1 - W_2) was considered as the weight of crude fibre in the moisture and fat free sample. The content of crude fibre in sample was calculated by using following formula.

$$\text{Crude fibre content g/100g} = \frac{W_2 - W_3}{W_1} \text{X } 100$$

Where,

W_1 - Weight of sample

W_2 - Weight of crucible with residue

W_3 - Weight of crucible with ash

4. Estimation of total mineral content

The total minerals of selected samples were estimated by the ashing methods of A.O.A.C. (1975)

Procedure

Three samples of highly accepted herbomix weighing 50 gm were taken in silica crucibles which were heated previously at 100^0 C and cooled. The crucible were placed on a clay pipe triangle and were heated on a low flame till the samples were completely charred. The charred samples were ignited by placing crucibles in muffle furnace for 5 hours at 600^o C. Thereafter crucibles were allowed to cool in desicator and weighed. The procedure was repeated till the consecutive weights obtained were concurrent and the ash was in grayish white colour. Total mineral content of samples was calculated by using the formula.

$$\text{Total mineral content of the samples (\%)} = \frac{W_3 - W_1}{W_2} \text{ X } 100$$

Where,

W_3 - Weight of Crucible with ash

W_1 - Weight of crucible

W_2 - Weight of sample

5. Determination of CHO content (NIN,1983)

The content of carbohydrate in the selected samples was obtained by subtracting from 100, the sum of value of moisture, protein, fat, ash and crude fibre content per 100 gm of the sample.

Carbohydrate = 100 – (Moisture + Protein + Fat + Ash + Crude fibre)

7 Determination of Calcium content

7.1 Preparation of reagents

7.1.1 4 N Sodium hydroxide

It was prepared by dissolving 160 gm of sodium hydroxide (NaOH) in glass distilled water and then volume was made up to 100ml.

7.1.2 Ammonium purpurate indicator

0.5 gm of ammonium purpurate was through mixed with 100 gm of powered potassium sulphate.

7.1.3 Ethylene diamine tetra acetic acid (Versenate) solution (0.01N)

2.0 gm of ammonium dihydrogen ethylene diamine tetra acetate and 0.05 g of magnesium chloride hexahydrate were dissolved in water and made the volume up to 100ml

Procedure for ash solution

5 gm of food sample is weighed accurately in a crucible charred and cooled in the desicator. The sample is ignited in muffle furnace at 600° C until light gray coloured ash is obtained. It is cooled and after cooling 5 ml of HCL is added by rinsing the upper portion of crucible and is evaporated to dryness an a steam water bath. The residue is dissolved by adding 2 ml of HCL. The crucible is rinsed by adding water and the crucible content is filtered into 100 ml volumetric flask after that it is cooled and diluted to volume.

Pipette 10 ml of diguested solution in china dish china dish, add 10 drops of 4N NaoH solution. Add approximately 50 mg of purpurate indicator, SHr with the help of glass rod and tritate with 0.1 N versenate solution till the

colour gradually changes from orange red to violet (purple). EDTA solution with a blank tritation.

Calculation

Volume of 0.01 N EDTA solution used = X ml

$$\text{Calcium (me/lit)} = \frac{\text{X ml x 0.01 N x 1000}}{\text{ml of aliquote}}$$

8. Determination of copper, zinc, iron and manganese

The trace elements (copper, zinc, iron and manganese) from the ash solution of the samples of herbal composite were estimated by atomic absorption spectrophotometer (Perkin R. Elmer Model 3110). The aliquots of each solution were fed to atomic absorption spectrophotometer through a capillary and reading were obtained directly in ppm.