

ORGANIC FEED FORMULATION INCORPORATING PROBIOTICS FOR DOMESTIC RODENTS

Submitted in partial fulfillment of the requirements for the Award of
Bachelor of Technology Degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A" by NAAC
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119**

April 2022



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **A.M. KAAVIYAA (38230018)** who carried out the project entitled **“ORGANIC FEED FORMULATION INCORPORATING PROBIOTICS FOR DOMESTIC RODENTS”** under our supervision from December 2019 to April 2020.

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DECLARATION

I **A.M. KAAVIYAA (38230018)** hereby declare that the Project Report entitled **“ORGANIC FEED FORMULATION INCORPORATING PROBIOTICS FOR DOMESTIC RODENTS”** doneby me under the guidance of **Dr. Masilamani Selvam**, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Domestic rodents mostly live on standard pellet mixes balanced for specific species nutritional needs, but the ration is not the only way to go. Many people have commercially developed food but most of them are made up of artificial and chemical substances which are harmful to them. In addition, they are costly and has side effects. This studies main source is industrial waste and probiotics. Usage of industrial food waste will be beneficial as it is readily available, cost effective and eco-friendly. Probiotics are microorganisms that naturally lives in the body of organism, it is composed of good microorganisms that helps keep the host body healthy and working well. Probiotics help restore the balance of natural flora and make it less possible for hamster to get ill in the first year. Proliferative ileitis or wet tail is a serious gastrointestinal infection which can be life threatening, which can be cured with balance diet and nutritious food. After collecting raw materials and extraction, an organic feed product is designed in a desirable form for domestic rodents. A series of tests such as anti-hemolytic, anti-oxidant, anti-bacterial, anti-inflammatory, anti-fungal, qualitative and quantitative phytochemical analysis, test for vitamins, nutritional analysis were performed for the formulated feed. Using the above combination of extracts an organic feed product is designed by incorporating probiotics in a desirable form for domestic rodents by trial and error method. This project main objective is to formulate organic feed for domestic rodents incorporated with probiotics as a treatment food for better reproduction, weight gain, bone health, overall body immunity and treatment for rodent specific diseases to overcome the disadvantages of existing commercial rodent food and supplements

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LIST OF SYMBOLS AND ABBREVIATIONS

pH	Power of Hydrogen ions
Nm	Nanometer
R _f	Retention Factor
TLC	Thin Layer Chromatography
PDA	Potato Dextrose Agar
NA	Nutrient Agar
GIT	gastrointestinal tract
APP	apple peel polyphenolic extract
IgA	Immunoglobulin A
NFκB	Nuclear factor kappa B
MAPKs	Mitogen-activated protein kinase
CP	Citrus peel
CNS	central nervous system
DPPH	1,1-diphenyl-2-picrylhydrazyl
TPTZ	6-Tripyridyl-s-triazine
NSAID	Non-Steroidal Anti Inflammatory Drugs
PBS	Phosphate Buffered Saline
NBT	Nitroblue Tetrazolium
EDTA	Ethylenediamine tetraacetic acid
μg	microgram
ml	milliliter
μl	microliter
Hr	hour
Min	minute
G	gram
L	liter
%	percentage
°C	degree Celsius
Fe ³⁺	ferric

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CHAPTER-1

INTRODUCTION

1.1 PROBIOTICS

Probiotics are beneficial components of the microbiota that have been used for centuries because of the health benefits they confer to the host. Only recently, however, has the contribution of probiotics to modulation of immunological, respiratory, and gastrointestinal functions started to be fully appreciated and scientifically evaluated. (Behnsen J et al., 2013). They are well-known as “health friendly bacteria”, which exhibit various health beneficial properties such as prevention of bowel diseases, improving the immune system, for lactose intolerance and intestinal microbial balance, exhibiting anti-hypercholesterolemia and antihypertensive effects, alleviation of postmenopausal disorders, and reducing traveler’s diarrhea. (Shi LH et al.,2016) In domestic rodents, probiotics have been shown to promote growth, improve the efficiency of feed utilization, modulate the gastrointestinal ecosystem, stimulate the immune system and protect the host from gastrointestinal tract (GIT) diseases. Therefore, probiotics provide a potential alternative strategy to in-feed antibiotics (Gaggia F et al.,2010), probiotics have been proposed as a means of promoting animal health but problems with their use has been reported. Research has demonstrated that bioactive molecules produced during the growth of certain probiotics interfere with bacterial cell-to-cell communication, which consequently results in an attenuation of virulence in a number of pathogens, including E. coli. (Nordeste R et al,2017)

1.1.1 LACTOBACILLUS

Lactobacillus is a genus of Gram-positive, rod-shaped, non-spore-forming bacteria. Lactobacilli are members of the lactic acid bacteria. They are strictly fermentative, aerotolerant or anaerobic, aciduric or acidophilic, and have complex nutritional requirements like carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, vitamins. (Tannock GW,2004)

1.1.2 LACTOBACILLUS REUTERI

Lactobacillus reuteri is a member of the normal microbial community of the gut in humans and animals. This organism produces antibiotic compounds, such as reutericin and reuterin, which have inhibitory effects on pathogenic microorganisms. In addition, *Lactobacillus reuteri* is commonly used as a probiotic to maintain the balance of the gut microbial flora and stimulate the intestinal immune system. , *L. reuteri* can benefit the host immune system. For instance, some *L. reuteri* strains can reduce the production of pro-inflammatory cytokines while promoting regulatory T cell development and function. the ability to strengthen the intestinal barrier, the colonization of *L. reuteri* may decrease the microbial translocation from the gut lumen to the tissues. Microbial translocation across the intestinal epithelium has been hypothesized as an initiator of inflammation. Therefore, inflammatory diseases, including those located in the gut as well as in remote tissues, may be ameliorated by increasing the colonization of *L. reuteri*. (Mu Q et al.,2018)

1.2 TARGETED DISEASE

Proliferative ileitis is a serious infectious disease of hamsters and usually results in high morbidity and mortality. The disease is characterized clinically by diarrhea in weanling hamsters and pathologically by segmental proliferative lesions of the epithelium of the terminal ileum. (Miedel EL and Hankenson FC,2015).

Hamsters usually die 24–48 hours after clinical signs of proliferative ileitis are noted, and deaths may occur in animals without premonitory signs. Early signs include lethargy, anorexia, irritability, ruffled hair coat, and rapid weight loss. As the disease progresses, there is the appearance of a fetid, watery diarrhea which causes moist matted fur on the perineum, tail, and ventral abdomen (Frisk et al,2012)

1.3 ORGANIC FEED FOR DOMESTIC RODENTS

1.3.1 *MUSA ACUMINATE*

Common Name	Banana
Kingdom	Plantae

Class	Liliopsida
Order	Zingiberales
Family	Musaceae
Genus	<i>Musa</i>
Species	<i>M. acuminata</i>

Table 1.1 TAXONOMY OF MUSA ACUMINATE



Fig 1.1 MUSA ACUMINATE

banana contains several bioactive compounds, such as phenolic, carotenoids, biogenic amines and phytosterols, which are highly desirable in the diet as they exert many positive effects on health and well-being. Many of these compounds have antioxidant activities and are effective in protecting the body against various oxidative stresses. (Singh B et al.,2016)

1.3.2 BRASSICA OLERACEA

Common Name	Cabbage
Kingdom	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family:	Brassicaceae
Genus:	<i>Brassica</i>
Species:	<i>B. oleracea</i>

Table 1.2 TAXONOMY OF BRASSICA OLERACEA



Fig 1.2 BRASSICA OLERACEA

Cabbage, which belongs to the family of Cruciferae, is one of the most important dietary vegetables (Zhang N et al.,2021). Cabbage is rich in various nutrients, including calcium, proteins, and vitamins C and E and contains various bioactive compounds with pharmacological properties, such as luteolin, myricetin, quercetin, and polyphenols (Yang and Dong Kwon, 2021)

1.3.3 BRASSICA OLERACEA VAR. GEMMIFERA

Common name	Brussel sprouts
Kingdom	Plantae
Phylum	Spermatophyta
Class	Dicotyledonae
Order	Capparidales
Family	Brassicaceae
Genus	<i>Brassica</i>
Species	<i>Brassica oleracea var. gemmifera</i>

Table 1.3 TAXONOMY OF BRASSICA OLERACEA VAR. GEMMIFERA



Fig 1.3 *BRASSICA OLERACEA VAR. GEMMIFERA*

Brussels sprout (*Brassica oleracea* var. *gemmifera*) is a cruciferous vegetable and an important rich source of sulfur-containing compounds known as glucosinolate (G. Roger Fenwick et al., 1983). Recent studies have reported that cruciferous vegetables including Brussels sprouts may protect humans against oxidative stress, several cancers, cardiovascular disease, diabetes, and hypertension as well as lower cholesterol levels. (Hwang and Eun-Sun, 2017)

1.3.4 *VITIS CALIFORNICA*

Common Name	Grapes
Kingdom	Plantae
Class	Magnoliopsida
Order	Vitales
Family	Vitaceae
Genus	<i>Vitis</i>
Species	<i>californica</i>

Table 1.4 TAXONOMY OF *VITIS CALIFORNICA*



Fig 1.4 VITIS CALIFORNICA

grape skin has a particularly high content of resveratrol (Frederiksen et al., 2007). Resveratrol is a phytoalexin produced by plants that are under attack. It has been reported to produce a variety of pharmacological effects. These effects contain anti-oxidant, anti-cancer, and anti-inflammatory properties (Bralley EE et al,2007)

1.3.5 SPINACIA OLERACEA

Common Name	Spinach
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Amaranthaceae
Genus	<i>Spinacia</i>
Species	<i>S. oleracea</i>

Table 1.5 TAXONOMY OF SPINACIA OLERACEA



Fig 1.5 SPINACIA OLERACEA

Spinach (*Spinacia oleracea* L.) is widely regarded as a functional food due to its diverse nutritional composition, which includes vitamins and minerals, and to its phytochemicals and bioactives that promote health beyond basic nutrition. Spinach-derived phytochemicals and bioactives are able to scavenge reactive oxygen species and prevent macromolecular oxidative damage, modulate expression and activity of genes involved in metabolism, proliferation, inflammation, and antioxidant defense (Roberts JL and Moreau R, 2016)

1.3.6 CITRUS SINENSIS

Common Name	Orange
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	<i>Citrus</i>
Species	<i>sinensis</i>

Table 1.6 TAXONOMY OF CITRUS SINENSIS



Fig 1.6 CITRUS SINENSIS

Orange contains an array of potent antioxidants including flavonoids (hesperetin and naringenin predominantly as glycosides), carotenoids (xanthophylls, cryptoxanthins,

carotenes), and vitamin C in addition to other beneficial phytochemicals, such as folate which are essential for health (Franke AA et al,2005)

1.3.7 MALUS DOMESTICA

Common Name	Apple
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Rosales
Family	Rosaceae
Genus	<i>Malus</i>
Species	<i>M. domestica</i>

Table 1.7 TAXONOMY OF MALUS DOMESTICA



Fig 1.7 MALUS DOMESTICA

apples possess rich contents of polyphenols, which are divided into several groups including hydroxybenzoic acids, hydroxycinnamic acids and their derivatives, flavonols, dihydrochalcones, anthocyanids, monomeric flavanols and oligomeric flavanols (Kalinowska et al., 2014). Due to the high nutraceutical values and various polyphenols of apples, apples have exhibited beneficial effects on the health against cancer, asthma and pulmonary dysfunction, cardiovascular diseases, Alzheimer's disease, decline of normal aging, weight management and diabetes (Hyson, 2011)

1.8 PHOENIX DACTYLIFERA

Common Name	Dates
Kingdom	Plantae
Phylum	Tracheophytes
Order	Arecales
Family	Arecaceae
Genus	<i>Phoenix</i>
Species	<i>P. dactylifera</i>

Table 1.8 TAXONOMY OF PHOENIX DACTYLIFERA



Fig 1.7 PHOENIX DACTYLIFERA

date seed contains protocatechuic acid, caffeoylshikimic acid and vanillic acid, that could potentially prevent the progression of neurodegenerative diseases, acting through their antioxidant properties. (Dallagi Y et al.,2020)

CHAPTER-2

LITERATURE SURVEY

Abdelkader Mezaini et al., (2010) investigated that the study of lactic acid bacteria strain was screened for the production of antibacterial substances active against some harmful bacteria. The inhibitory mechanism was investigated. Antibacterial activity of probiotics against indicator bacteria is a good probiotic property. Transfer of these antimicrobial activities is beneficial for the maintenance of the intestinal microflora.

pankaja s and Chede, (2013) reported that *Citrus Sinensis* peel was screened for its phytochemical composition. The aqueous as well as the ethanolic extracts of the peel revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, terpenoids, steroids, and amino acids whereas the saponins are present only in the ethanolic extract. *Citrus Sinensis* peel, limonene, is now used as a significant chemopreventive agent (Crowell,1999) with a potential value as a dietary anti-cancer tool in humans. Orange Peel is medically used against fungi.

Jia et al (2017), investigated the preventive effects of Red Fuji apple peel polyphenolic extract (APP) on vascular endothelial dysfunction and liver injury in mice fed a high choline diet. It indicated that the polyphenolic extract from apple peel might be regarded as a preventive and therapeutic product for the amelioration of HC diet-induced vascular dysfunction, Gastrointestinal Diseases and hepatic injury

Hemarajata et al., (2013) in this study it is reported that Probiotic mechanisms in the human gastrointestinal tract. Probiotics may manipulate intestinal microbial communities and suppress growth of pathogens by inducing the host's production of β -defensin and IgA. Probiotics may be able to fortify the intestinal barrier by maintaining tight junctions and inducing mucin production. Probiotic-mediated immunomodulation may occur through mediation of cytokine secretion through signaling pathways such as NF κ B and MAPKs, which can also affect proliferation and differentiation of immune cells (such as T cells) or epithelial cells.

Balwinder Singh et al., (2020) reported that Citrus peel (CP) forms around 40-50% of the total fruit mass but it is generally a waste. The source of naturally occurring

health enhancing compounds, particularly phenolic compounds and carotenoids. Phenolic compounds in CP mainly comprise phenolic acids (primarily caffeic, p-coumaric, ferulic and sinapic acid), flavanones (generally naringin and hesperidin) and polymethoxylated flavones (notably nobiletin and tangeretin). Phenolic compounds present in citrus peel act as antioxidants and protect the cells against free radical damage as well as help in reducing the risk of many chronic diseases. Thus, the results showed that the peels of citrus fruits can be used as a source of functional compounds and preservatives for the development of food products. They also have health- promoting activities.

Rahayu et al., (2007) stated that Lactic acid bacteria are one of the bacteria that have potential use as probiotics. Lactic acid bacteria can survive by forming colonies of the intestine and can also produce lactic acid and bacteriocins. Lactic acid bacteria consist of several genera of bacteria belonging to the family of Firmicutes Lactic acid bacterial isolates of the genus *Lactobacillus* are generally potential as probiotic agents which beneficial to human and animal health

Kumar H et al.,(2020) reported that a large amount of peel waste is generated from fruit and vegetable-based industries and household kitchen and has led to a big nutritional and economic loss and environmental problems. Processing of fruits and vegetables alone generates a significant waste, which amounts to 25–30% of the total product. Most common wastes include pomace, peels, rind and seeds, which are highly rich in valuable bioactive compounds such as carotenoids, enzymes, polyphenols, oils, vitamins and many other compounds. These bioactive compounds show their application in various industries such as food to develop edible films, food industries for probiotics and other industries for valuable products.

Fedorova et al., (2007) the role of the omega-3 fatty acids in the regulation of cognitive functions, locomotor and exploratory activity and emotional status in rodents. there is some evidence of an enhanced vulnerability to stress of n-3 fatty acid deficient animals and this factor can influence performance

Bravo JA et al., (2010) There is increasing evidence suggesting an interaction between the intestinal microbiota, the gut, and the central nervous system (CNS) in what is recognized as the microbiome–gut–brain axis. Studies in rodents have

implicated dysregulation of this axis in functional bowel disorders, including irritable bowel syndrome. Indeed, visceral perception in rodents can be affected by alterations in gut microbiota

Di Cerbo A et al., (2016) lactobacilli have been used as an effective therapy for treatment of several pathological conditions displaying an overall positive safety profile. In vitro and in vivo investigations have shown that prolonged lactobacilli administration induces qualitative and quantitative modifications in the human gastrointestinal microbial ecosystem with encouraging perspectives in counteracting pathology-associated physiological and immunological changes.

Verna EC and Lucak, (2010) S Probiotics are being used with increasing frequency as a treatment for several medical conditions, such as allergic diseases (atopic dermatitis, possibly allergic rhinitis), bacterial vaginosis, urinary tract infections, and prevention of dental caries or respiratory infections. Probiotics are used as a treatment for a variety of gastrointestinal (GI) disorders. In this review, the historical perspectives, proposed mechanisms of action, formulations and delivery systems, safety, and specific GI disorders for which probiotics have been used are discussed.

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM

1. To formulate organic feed for domestic rodents incorporated with probiotics as a treatment food for better reproduction, weight gain, bone health, overall body immunity and treatment for rodent specific diseases
2. To analyze the bio potential activities of the formulated organic feed

3.2 OBJECTIVE

1. collection of source Extraction of above source by aqueous extraction method for further analysis
2. To perform qualitative phytochemical analysis
3. To perform quantitative phytochemical analysis
4. Analyze the bio potential activity of the source by using the aqueous extraction viz.,
 - Anti-oxidant activity.
 - Anti-inflammatory activity.
 - Anti-bacterial activity
 - Anti-diabetic activity
5. Collection of raw materials and drying them for product formulation and trails
6. To Formulate organic feed and incorporating probiotics by spray drying method
7. To check the nutrition profile for the organic feed

CHAPTER-4

MATERIALS AND METHODS

4.1 COLLECTION OF SAMPLE

The *Musa acuminata*(peel), *Brassica oleracea*, *Brassica gemmifera*, *Vitis californica*, *Spinacia oleracea*, *Citrus sinensis*, *Phoenix dactylifera*, *Malus domestica* were collected from nellai deen store from adambakkam in Chennai district ,Tamil Nadu. . The sources were washed with tap water and then followed by distilled water. The sources were separated by cutting them into smaller pieces and smashed properly using mortar and pestle and transferred into conical flasks and it was used for the extraction process.

4.2 ALCOHOL EXTRACTION

The *Musa acuminata*(peel), *Brassica oleracea*, *Brassica gemmifera*, *Vitis californica*, *Spinacia oleracea*, *Citrus sinensis*, *Phoenix dactylifera*, *Malus domestica* are crushed with mortar and pestle. Methanol was added till the content sinks and covered with aluminum foil. Incubate at room temperature for 72 hrs. Extracts were then further transferred into sterile bottles for further use

4.3 IN VITRO ANTIOXIDANT ASSAYS

4.3.1 DPPH[•] RADICAL SCAVENGING ACTIVITY

Scavenging of DPPH free radicals is one of the popular antioxidant methods. DPPH radical scavenging assay is a decolorization assay that will measure the capacity of antioxidants to directly scavenge DPPH[•] radicals (Kedare and Singh, 2011). The ability of aqueous extract to scavenge free radicals was assessed by the DPPH method. The maximum DPPH[•] radical scavenging activity of methanol extract was 66.99±0.75% at 300 µg/mL concentration (Table 3, Fig 3) and the IC₅₀ was 157.52 µg/mL concentration. It was compared with the standard of ascorbic acid (IC₅₀ = 11.98 µg/mL concentration). In this method, the purple colour 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was reduced to yellow colour 1,1-diphenyl-2-picrylhydrazine, and the reducing capacity increases with increasing concentration of the extract.

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

4.3.2 PHOSPHOMOLYBDENUM REDUCTION ASSAY

The total antioxidant activity of aqueous extract was measured by phosphomolybdenum reduction assay method. In this method, Mo (VI) ion reduced to Mo (V) by the extract and the formation of green phosphate/Mo (V) complex at acidic pH (Loo et al., 2008). The maximum phosphomolybdenum reduction was 79.86±0.87% at 300 µg/mL concentration and the RC₅₀ was 51.06 µg/mL concentration. It was compared with the standard ascorbic acid (RC₅₀ = 6.34 µg/mL concentration). Phosphomolybdenum reduction assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant, and molybdenum ligand. It involves thermally generating auto-oxidation during the prolonged incubation period at a higher temperature.

$$\% \text{ of phosphomolybdenum reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

4.3.3 Fe³⁺ REDUCING ASSAY

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Ferric reducing antioxidant assay was originally developed by Benzie and strain to measure reducing power in plasma, but the assay subsequently has also been adapted & used for the assay of antioxidant in botanicals. The reaction measures reduction of Ferric 2, 4, 6- tripyridyl-s- triazine (TPTZ) to a Navy blue colored product. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols.

One mL of extract with different concentrations (20 - 120 µg/mL) and make up 1ml with methanol was mixed with 1 mL of 1% (w/v) potassium ferricyanide [K₃Fe (CN)₆] solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixtures were then incubated at 50°C in a water bath for 20 minutes. 500 µL of 10% (w/v) trichloroacetic acid solution was added to each mixture followed by 1 mL of 0.1% (w/v) freshly prepared FeCl₃ solution was added and shaken well. The absorbance was measured at 700 nm (Benzie et al.,1996).

The percentage of inhibition was calculated using the following formula:

$$\% \text{ of } \text{Fe}^{3+} \text{ reducing assay} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

4.4 ANTIDIABETIC ACTIVITY

4.4.1 ALPHA AMYLASE ENZYME INHIBITION ASSAY

α- amylase enzyme inhibition assay was carried out based on the starch-iodine test (Xiao et al., 2006). The total assay mixture was composed of various concentration (20-120 µg/mL) of aqueous extract, 10 µL of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and was incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction set and incubated at 37°C for 60 min. One hundred µL of 1 M HCl was added to stop the enzymatic reaction and followed by 200 µL of iodine reagent (5 mM I₂ and 5 mM KI) was added. The colour change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did not contain any extract. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor indicating that starch is completely hydrolyzed by α-amylase.

$$\% \text{ of } \alpha\text{- amylase enzyme inhibition} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

4.5 ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory is the property of treatment to reduce inflammatory. Inflammation is the body attempt at self-protection to remove harmful stimuli, including damaged cells, pathogens and begin the healing process. Many bioactive compounds have anti-inflammatory activities (FatmaAlhakmani.et.al 2013).

4.5.1 MEMBRANE STABILIZATION ASSAY

4.5.1.1 PREPARATION OF RED BLOOD CELLS (RBCs) SUSPENSION

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 min and discard the pale yellow plasma. Then, about 10 mL of PBS was poured into centrifuge tube, centrifuged at 3000 rpm for 5 min, discard and discard the supernatant and repeat the washing process once again. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH_2PO_4 , 1.15 g of Na_2HPO_4 and 9 g of NaCl in 1 litre of distilled water. The reconstituted red blood cells (resuspended supernatant) were used as such normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

4.5.1.2 HEAT INDUCED HAEMOLYSIS

The reaction mixture (2 mL) consisted of 1 mL of different concentrations of aqueous extract (20-120 $\mu\text{g/mL}$) and 1 mL of 10% RBCs suspension, instead of test sample only saline was added to the control test tube (Shinde et al., 1989). All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The

reaction mixture was centrifuged at 3000 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. Aspirin was used as a standard drug.

The percentage inhibition of haemolysis was calculated as follows:

$$\% \text{ of hemolysis inhibition} = \frac{\text{Control} - \text{Sample} \times 100}{\text{Control}}$$

4.6 ANTIBACTERIAL ACTIVITY

The microorganisms of Gram-positive bacteria such as *Staphylococcus aureus*, and Gram negative bacteria *Escherichia coli* and *Pseudomonas argenosa* were used for the evaluation of the antibacterial activity.). The antibacterial activity may be due to the presence of phenolic compounds, terpenoids, which inhibit bacterial cell wall biosynthesis by binding to a highly conserved motif of lipid II and lipid III, two key precursors of bacterial cell-wall polymers as peptidoglycan and teichoic acid that adversely affect the growth of microbes (Obeidat et al., 2012).

4.6.1 AGAR WELL DIFFUSION METHOD

Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast extract-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g). The required amount of ingredients was weighed and suspended in the required volume of 100 mL of distilled water and then autoclaved at 15 lbs and 121°C for 15 min. The hot medium was poured in sterile petri plates which were kept in the aseptic Laminar chamber. The medium was allowed to solidify for 15 min.

Antibacterial activity of aqueous extract was carried out using agar well diffusion method (Kubo et al., 2002). The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which are previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The aqueous extract was then poured into each well containing 250, 375, and 500 µg/mL concentrations. All the plates with extract

loaded wells were incubated at 37°C for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was used as the positive control.

4.7 PHYTOCHEMICAL ANALYSIS

Phytochemicals are naturally present in the plants and show biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. The secretion of these compounds is varying from plant to plant, some produce more and some produce in minimal quantities. Sometimes they can be harmful and sometimes they can be very helpful. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of hypertension, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims by the US Food and Drug Administration.

4.8 QUALITATIVE ANALYSIS

4.8.1 DETECTION OF ALKALOIDS (HAGER'S TEST)

It possesses anti-inflammatory, anti-cancer, analgesic, local anesthetic & pain relief, neuropharmacologic activities. Alkaloids are formed as end products of detoxification reactions such as secondary metabolites. They serve as a regulatory growth factor in plants. They also serve as reserve substances capable of supplying nitrogen or other elements necessary to the plant's economy. Alkaloids like morphine are used for pain relief but its usefulness is limited because of its addictive property.

To the extract, few drops of concentrated hydrochloric acid were added by the sides of the test tube, followed by a few drops of saturated picric acid solution.

4.8.2 DETECTION OF PHENOL (FOLIN CIOCALTEU)

Anti-aging, anti-inflammatory, antioxidant & anti-proliferative agent properties.

There are relevant anti-oxidant properties to counter oxidants.

To the extract, 200µl of neutral ferric chloride solution was added and shaken well.

4.8.3 DETECTION OF GLYCOSIDES (RAYMOND'S TEST)

Flavonoid C-glycosides showed significant antioxidant activity, anti-cancer & anti-tumor activity, hepatoprotective activity, anti-inflammatory activity, anti-diabetes activity, antiviral activity & other biological effects.

To the extract, 100l methanol was added followed by Sodium nitroprusside solution with a few drops and 3-5 drops of Pyridine was added and shaken well and few drops of concentrated Sulphuric acid was added.

4.8.4 DETECTION OF TERPENOIDS (SALKOWSKI TEST)

Terpenoids have been found useful in prevention & therapy of several diseases including cancer & also have anti-fungal, anti-microbial, anti-parasitic, anti-inflammatory & immune- modulatory properties. It is also used to enhance skin penetration.

To extract 2 ml of chloroform and followed by Concentrated H₂SO₄ (3ml) was carefully added.

4.8.5 DETECTION OF FLAVONOIDS

Flavonoids possess a number of medicinal benefits, including anti-cancer, anti-oxidant, anti-inflammatory & antiviral properties. It helps regulate cellular activity & fight off free radicals that cause oxidative stress on your body.

To the extract, a few drops of 1% NaOH solution were added.

4.8.6 DETECTION OF TANNINS

They are antioxidants which scavenge and neutralize free radicals and combating oxidative stress. They contain anti-cancer/ cancer-preventing properties.

To the extract diluted with 2ml distilled water, a few drops of lead acetate solution was added.

4.8.7 DETECTION OF SAPONINS

It decreases blood lipids, lowers cancer risks, and lowers blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans and as an antidote against acute lead poisoning.

To extract it is diluted with 5ml distilled water. The suspension was shaken in a graduated cylinder for 15 minutes.

4.8.8 DETECTION OF STEROIDS

Increased red blood cell production, enhanced recovery from workout and injury, improved bone mineral density. It is mainly used to decrease pain and increase function

To the extract, a few drops of acetic anhydride was added, and shaken well followed by a few drops of concentrated sulphuric acid was added along the sides of the test tube carefully.

4.9 QUANTITATIVE ANALYSIS

4.9.1 ESTIMATION OF FLAVONOIDS

To 500 µl of extract from 1mg/ml solution 500µl methanol was added. Then 1ml of 5% sodium nitrate solution was added. To this 1ml of 10% aluminium chloride solution was also added and Shaken well and add 1ml of sodium hydroxide and was incubated at room temperature for 30 minutes. Centrifuged at 3000rpm for 10 minutes and collect the supernatant. Absorbance of supernatant was taken at 570 nm.

4.9.2 ESTIMATION OF PHENOLS

To 100 µl of extract from (1mg/mL) solution 900 µl methanol was added. Then 1ml of 1:10 diluted Folin-Ciocalteu reagent was added. To this 1ml of 20% sodium carbonate solution was also added and Shaken well and then incubated at room temperature for 30 minutes in dark. Absorbance of supernatant was taken at 765 nm

4.9.3 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) was carried out for the aril aqueous extract of in Merck TLC aluminum sheets, silica gel 60 F254 (20 x 20 cm), preloaded plates. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 254 nm. The R_f values of the coloured spots were recorded. The ratio in which distinct bands appeared was optimized and R_f values were calculated.

Calculation of R_f value:

R_f value = Distance travelled by the solute

Distance travelled by the solvent

CHAPTER- 5

RESULTS AND DISCUSSION

5.1 FORMULATION OF ORGANIC FEED INCORPORATING PROBIOTICS USING SPRAY DRYING METHOD

The powdered source including *Musa acuminata* (peel), *Brassica oleracea*, *Brassica gemmifera*, *Vitis californica*, *Spinacia oleracea*, *Citrus sinensis*, *Phoenix dactylifera*, *Malus domestica* were collected and mixed with lukewarm water 0.05% of multivita fish mineral oil and groundnut oil was added for nutrition enrichment .the mixed source was then made into pellets. Probiotics was mixed with sterile water and incorporated to the feed by spray drying method

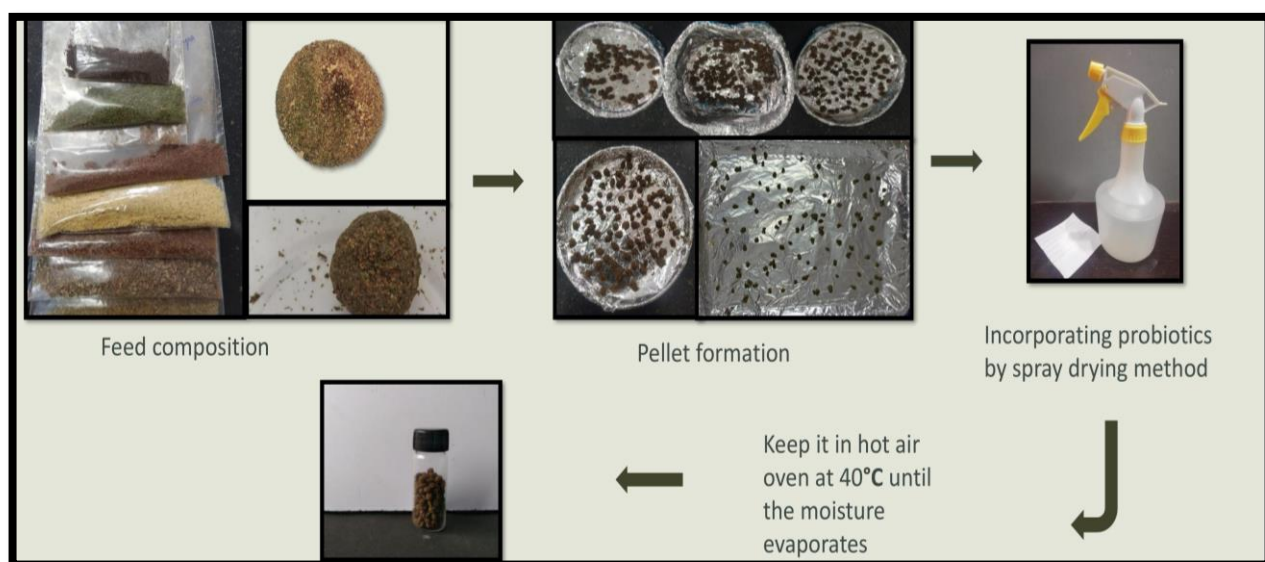


Fig 5.1 PRODUCT FORMULATION

5.2 IN VITRO ANTIOXIDANT ASSAYS

5.2.1 DPPH' RADICAL SCAVENGING ACTIVITY

The antioxidant activities of extract evaluated using DPPH method. DPPH was absorbed at 517nm. The inhibitory activity of extract as the concentration increases the inhibitory activity also increases. As shown in Table.5 For the DPPH radical

scavenging assay IC₅₀ values of 60µL is 49.72% is below 50% and 100µl ,120µl the inhibition is above 50%

S.NO	EXTRACT CONCENTRATION	OPTICAL DENSITY AT 517 NM	PERCENT INHIBITION
1.	Control	0.179	-
2.	20 µL	0.133	25.69%
3.	40 µL	0.125	30.16%
4.	60 µL	0.102	43.01%
5.	80 µL	0.090	49.72%
6.	100 µL	0.085	52.51%
7.	120 µL	0.072	59.77%

Table 5.1 DPPH[•] RADICAL SCAVENGING ACTIVITY OF EXTRACT

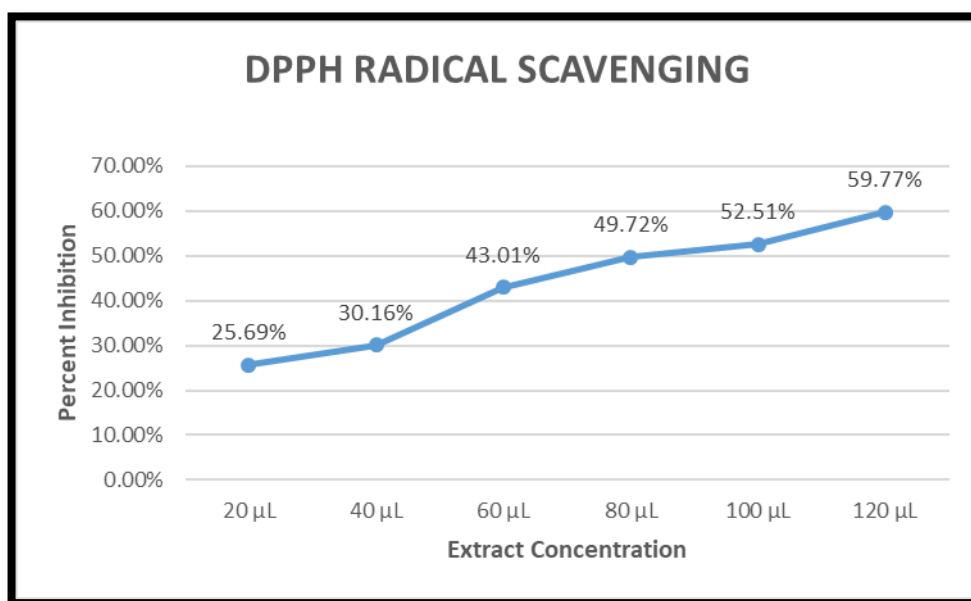


Fig 5.2 GRAPH OF DPPH[•] RADICAL SCAVENGING ACTIVITY OF EXTRACT

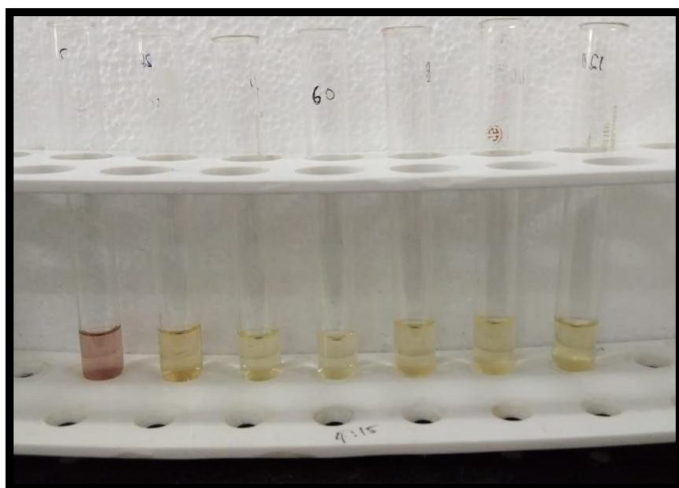


Fig 5.3 DPPH[•] RADICAL SCAVENGING ACTIVITY OF EXTRACT

5.2.2 PHOSPHOMOLYBDENUM REDUCTION ACTIVITY

The antioxidant activities of extract were evaluated using Phosphomolybdenum method. Phosphomolybdenum reducing was absorbed at 695nm. For the Phosphomolybdenum assay IC₅₀ values of 20μL, 40μL, 60μL, 80μL, 100μL and 120μl are above 50% which didn't show best results.

S.NO	EXTRACT CONCENTRATION	OPTICAL DENSITY AT 695 NM	PERCENT INHIBITION
1.	Control	0.135	-
2.	20 μL	0.350	61.42%
3.	60 μL	1.321	89.78%
4.	40 μL	1.458	90.74%
5.	80 μL	1.515	91.08%
6.	100 μL	1.744	92.25%
7.	120 μL	2.050	93.41%

Table 5.2 PHOSPHOMOLYBDENUM REDUCTION ACTIVITY OF EXTRACT

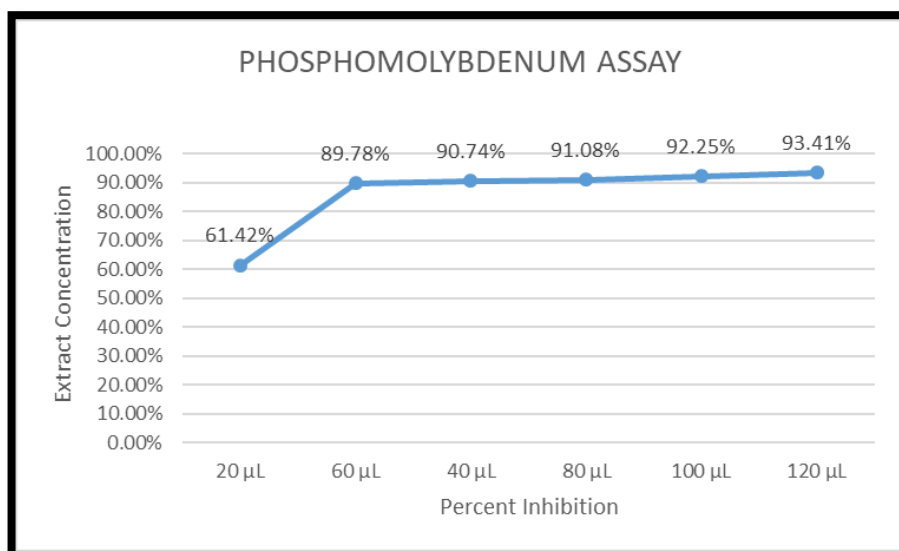


Fig 5.4 GRAPH OF PHOSPHOMOLYBDENUM REDUCTION ACTIVITY OF EXTRACT OF PERCENTAGE INHIBITION VS EXTRACT CONCENTRATION

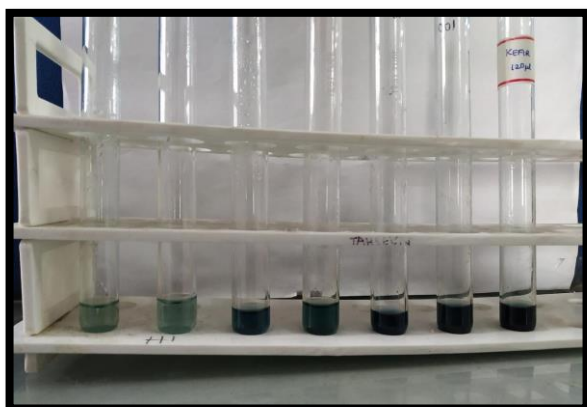


Fig 5.5 PHOSPHOMOLYBDENUM REDUCTION ASSAY

5.2.3 Fe³⁺ REDUCTION ASSAY

The antioxidant activities of extract were evaluated using Fe³⁺ Reducing method. Fe³⁺ Reducing was absorbed at 700nm. The inhibitory activity of extract as the concentration increases the inhibitory activity also increases. As shown in Table.4 For the Fe³⁺ Reduction assay IC₅₀ values of 120 µl is 32.02 are below 50%

S.NO	PLANT EXTRACT CONCENTRATION	OPTICAL DENSITY AT 700 NM	PERCENT INHIBITION
1.	Control	1.700	-
2.	20 μ L	1.763	3.57 %
3.	40 μ L	1.795	5.29%
4.	60 μ L	2.289	25.73%
5.	80 μ L	2.315	26.56%
6.	100 μ L	2.478	31.39%
7.	120 μ L	2.501	32.02%

Table 5.3 Fe^{3+} REDUCTION ASSAY

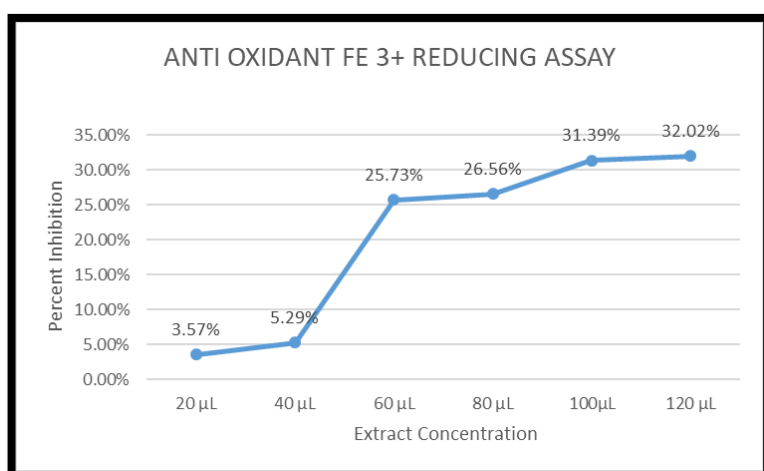


Fig 5.6 Fe^{3+} REDUCTION ASSAY GRAPH OF PERCENTAGE INHIBITION VS EXTRACT CONCENTRATION

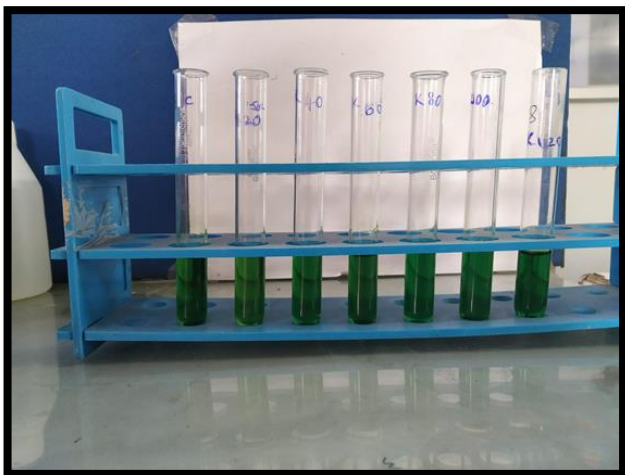


Fig 5.7 Fe³⁺ REDUCTION ASSAY

5.3 ANTIDIABETIC ASSAY

The antidiabetic activities of extract were evaluated using Alpha amylase enzyme inhibition assay and was absorbed at 540nm. The inhibitory activity of extract as the concentration increases the inhibitory activity also increases. As shown in Table.4 For the Alpha amylase enzyme inhibition assay IC₅₀ values of 120 μ L is 27.20% are below 50%

S.NO	PLANT EXTRACT CONCENTRATION	OPTICAL DENSITY AT 540 NM	PERCENT INHIBITION
1.	Control	0.760	-
2.	20 μ L	0.669	-
3.	40 μ L	0.776	2.60%
4.	60 μ L	0.808	5.94%
5.	80 μ L	0.907	16.20%
6.	100 μ L	0.951	20.08%
7.	120 μ L	1.044	27.20%

Table 5.4 ANTIDIABETIC ASSAY

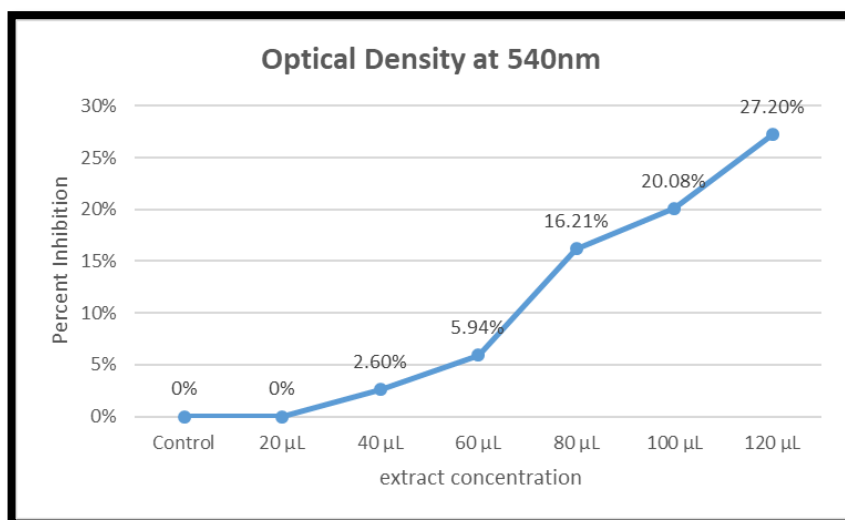


Fig 5.8 ANTIDIABETIC ASSAY GRAPH

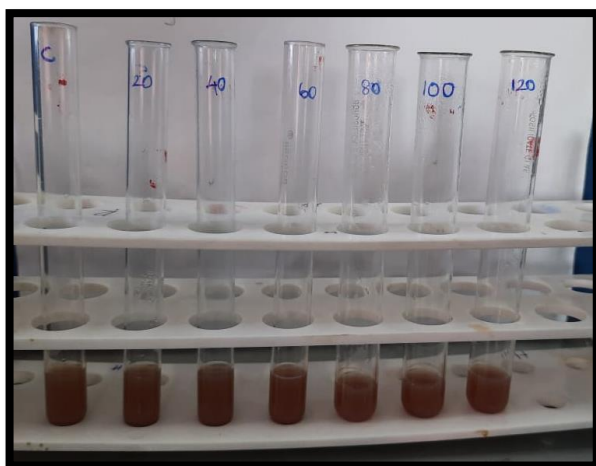


Fig 5.9 ANTIDIABETIC ASSAY

5.4 ANTI-INFLAMMATORY ASSAY

The Anti-inflammatory activities of extract evaluated using Anti-inflammatory assay. Absorbance at 560nm. The inhibitory activity of extract as the concentration increases the inhibitory activity also increases. As shown in Table.8 For the Anti-inflammatory assay IC₅₀ values of 120µL is 3.55% which are below 50% 100µL

S.NO	EXTRACT CONCENTRATION	OD DENSITY AT 370 NM	PERCENT INHIBITION
1.	Control	2.201	-
2.	20 μ L	2.240	1.74 %
3.	40 μ L	2.250	2.17%
4.	60 μ L	2.252	2.26%
5.	80 μ L	2.265	2.28%
6.	100 μ L	2.276	3.29%
7.	120 μ L	2.281	3.55%

Table 5.5 ANTI-INFLAMMATORY ASSAY

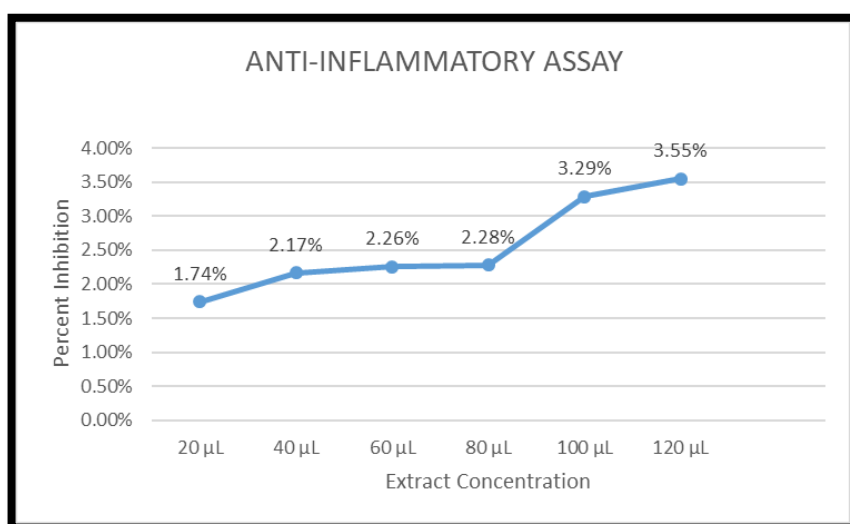


Fig 5.10 ANTI-INFLAMMATORY ASSAY GRAPH

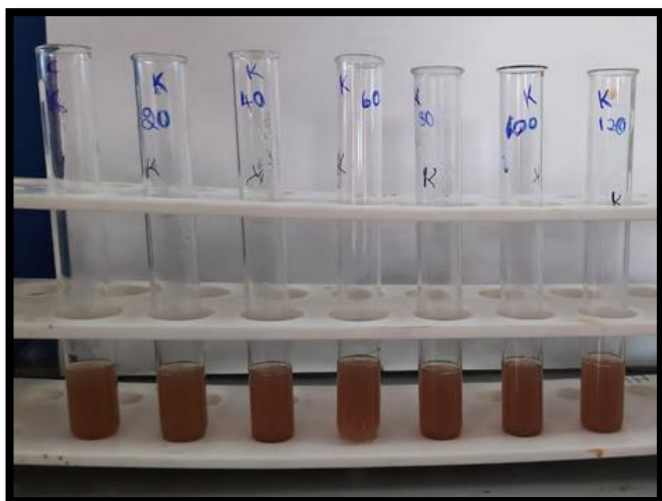


Fig 5.11 ANTI-INFLAMMATORY ASSAY

5.5 ANTIBACTERIAL ACTIVITY

The antibacterial sensitivity of the crude extract and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in Petri plates. The maximum zone of inhibition for *Escherichia coli* was 19 mm at 500 $\mu\text{g/mL}$ concentration. The antibacterial activity may be due to the presence of secondary metabolites such as phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth of microbes. plates which revealed that the organic feed had the ability to fight against infectious bacteria such *Escherichia coli* and *Staphylococcus aureus*.

NAME OF ORGANISMS	STANDARD	250 $\mu\text{g/ml}$	375 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$
Staphylococcus aureus (SA)	19mm	12mm	14mm	15mm
Escherichia coli	25mm	15mm	17mm	19mm

Pseudomonas aeruginosa	20mm	-	-	-
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Table 5.6 ANTI-BACTERIAL ACTIVITY

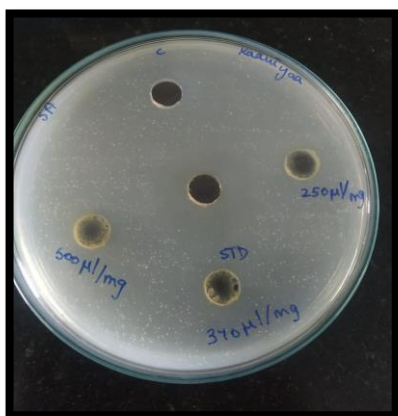


Fig 5.12 ANTI-BACTERIAL ACTIVITY OF STAPHYLOCOCCUS AUREUS(sa)



Fig 5.13 ANTI-BACTERIAL ACTIVITY OF ESCHERICHIA COLI

5.6 PHYTOCHEMICAL ASSAY

5.6.1 QUALITATIVE ANALYSIS

The qualitative phytochemical analysis of the combination of the extract exhibited the presence of the alkaloids, Terpenoids, Phenolic compounds, Flavonoids, Tannins, Glycosides and Steroid

S.NO	PHYTOCHEMICAL TEST	OBSERVATION	INFERENCE
1.	Alkaloids	Yellow precipitate	Present
2.	Terpenoids	Red ring formed	Present
3.	Steroids	Dark colour formed	present
4.	Phenolic compounds	Dark green colour	Present
5.	Flavonoids	Yellow colour formation	Present
6.	Tannins	White precipitate	Present
7.	Glycosides	Red brown colour	Present
8.	Saponins	Froth appeared	Present

Table 5.7 QUALITATIVE PHYTOCHEMICAL ASSAY

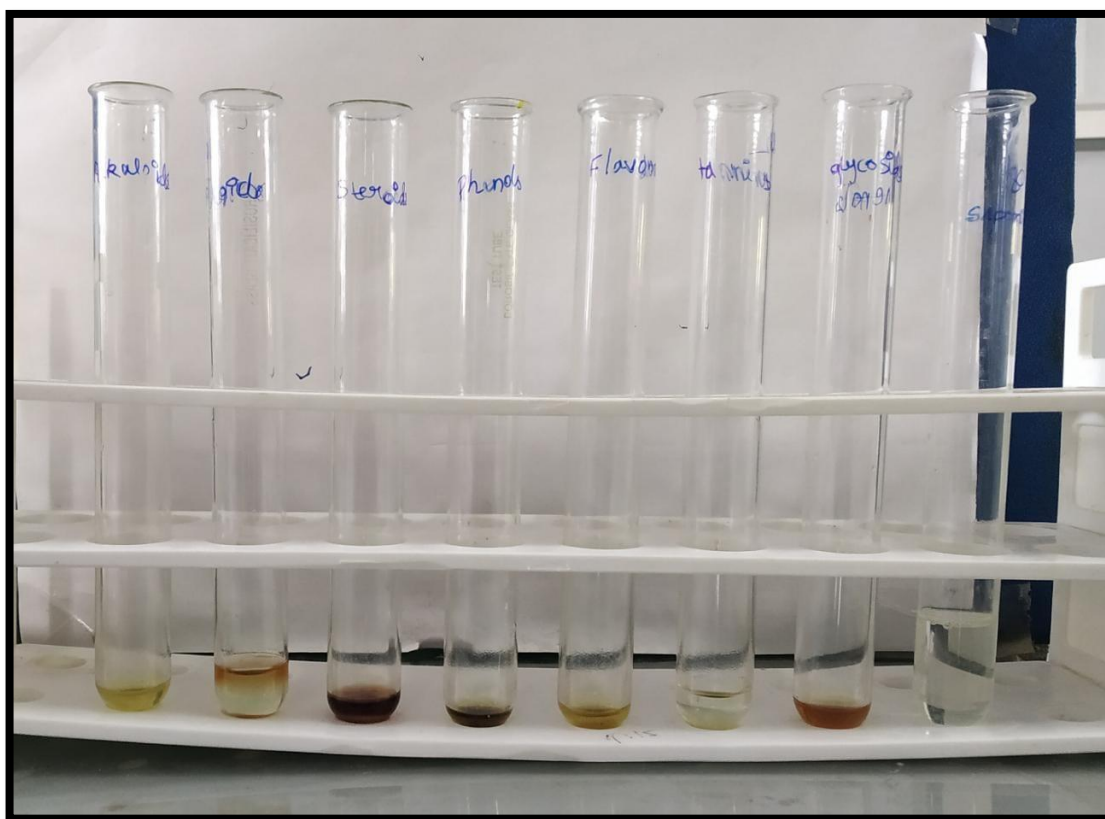


Fig 5.14 QUALITATIVE PHYTOCHEMICAL ANALYSIS

5.6.2 QUANTITATIVE ANALYSIS

5.6.2.1 ESTIMATION OF FLAVANOIDS

The total flavonoid compound of the combination extract was expressed as quercetin equivalent 55.39 $\mu\text{g}/\text{mg}$

S.NO	OD AT 570nm	QE ($\mu\text{g}/\text{mg}$)
1.	1.117	55.39 $\mu\text{g}/\text{mg}$

Table 5.8 ESTIMATION OF FLAVONOIDS

5.6.2.2 ESTIMATION OF PHENOL

The total phenolic compounds of the combination extract were expressed as Gallic acid equivalent 739.3 $\mu\text{g}/\text{mg}$

S.NO	OD AT 765nm	GAE ($\mu\text{g}/\text{mg}$)
1.	1.446	739.3 $\mu\text{g}/\text{mg}$

Table 5.9 ESTIMATION OF PHENOL

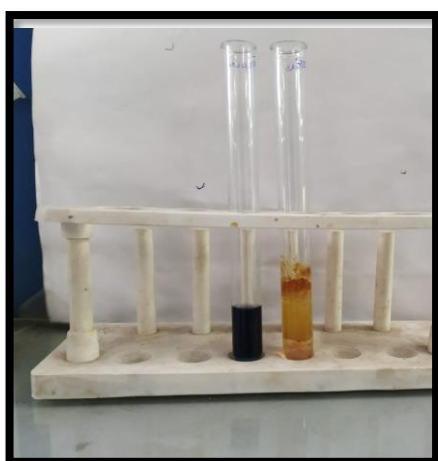


Fig 5.15 QUANTITATIVE ANALYSIS OF PHENOL AND FLAVONOIDS

5.7 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography analysis was carried out in the solvent system of

Methanol: Chloroform with the ratio of 1.5:0.5. The separated compounds in TLC were showed in Figure.

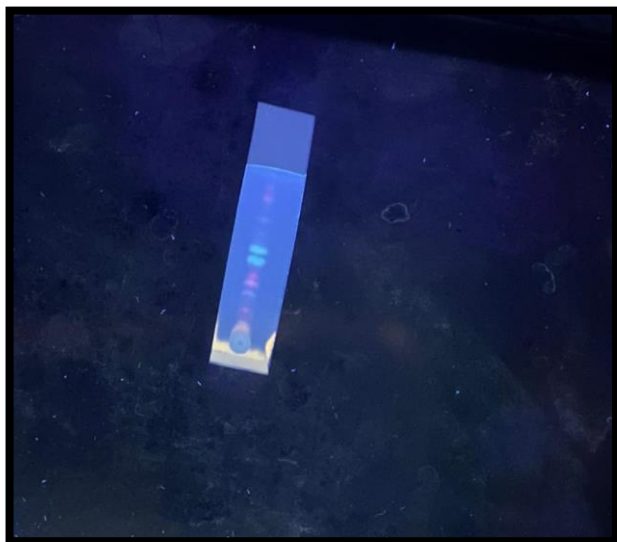


Fig 5.16 COMPOUNDS SEPARATED BY THIN LAYER CHROMATOGRAPHY

5.8 PRELIMINARY STUDIES

The preliminary studies conducted for three weeks revealed that organic feed incorporated with probiotics was slightly better than commercially available synthetic pellets

5.8.1 CONTROL

Breed: Syrian hamster

Age: 3 months

Color: caramel brown

Gender: female

WEEKS	FOOD	TAIL	NAIL	NOSTRIL	EYE	EXCREATA	WATER INTAKE	WEIGHT GAIN
1	10g of cabbage + 10g of carrot + 10g of chick pea skin	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	70.6g
2	10g of cabbage + 10g of beans+ 10g of carrot	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	72.3g
3	10g of cabbage + 10g of carrot + 10g of beans	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	74.2g

Table 5.10 PRELIMINARY STUDIES OF CONTROL ANIMAL

5.8.2 TEST

Breed: Syrian hamster

Age: 2 months

Color: mutated ash

Gender: male

WEEKS	FOOD	TAIL	NAIL	NOSTRIL	EYE	EXCREATA	WATER INTAKE	WEIGHT GAIN
1	10g of Brussel sprouts + 20g of formed product	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	48.6g
2	10g of brussel sprouts + 20g of formed product	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	52.4g
3	10g of apple peel + 20g of formed product	normal	pinkish white (healthy)	no water leak	no fluids leak	healthy	regular	56.8g

Table 5.11 PRELIMINARY STUDIES OF TEST ANIMAL

5.9 NUTRITION ANALYSIS

The nutrition analysis of the organic feed revealed that carbohydrate and protein content of the organic feed was 31.3 g and 63 g respectively.

5.9.1 ANALYSIS FOR THE VALUE AND THE NUTRITIONAL CONTENT IN THE ORGANIC FEED

SL.NO	TEST PARAMETER(S)	RESULTS
1	Protein	31.3
2	Carbohydrate	63
3	Fat	6.05

4	Energy	359
5	Moisture	10.97
6	Ash	6.66
7	Sodium as Na	272.2
8	Potassium as K	2161.20
9	Calcium as Ca	417.07
10	Magnesium as Mg	213.39
11	Phosphate	1346.64

Table 5.12 NUTRITION AND CHEMICAL COMPOSITION OF THE FEED

5.10 GAS CHROMATOGRAPHY–MASS SPECTROMETRY

GC/MS analysis indicated the presecence of organic compounds such as 4-Hydroxy-3-methoxybenzyl alcohol, Flavone, Methane.bis(p-methoxyphenyl), 2H-1-benzopyran-4 7-diol,3.4-dihydro-2-phenyl, n-Hexadecanoic acid, cis-11,12-Epoxytetradecen-1-ol, 1 –hexocosence which all possessing various biological function

sno	Retention time	Compound name	Molecular weight	Molecular formula	structure	Pharmacological action
1	10.8	4-Hydroxy-3-methoxybenzyl alcohol	154.1632	C ₈ H ₁₀ O ₃		anti-inflammatory and analgesic activity(Jang YW,2010)
2	13.0	Flavone	222.2387	C ₁₅ H ₁₀ O ₂		antioxidative activity, hepatoprotective, anti-inflammatory, and anticancer activities.(Kumar S et al.,2013)
3	13.77	Methane.bis(p-methoxyphenyl)	228.2863	C ₁₅ H ₁₆ O ₂		anti-inflammatory, antiarthritic activities(David G t al.,1972)
4	14.6	2H-1-benzopyran-4 7-diol,3,4-dihydro-2-phenyl	210.2711	C ₁₅ H ₁₄ O		antibacterial activity (Krishnaveni et al.,2015)
5.	15.25	n-Hexadecanoic acid	256.4241	C ₁₆ H ₃₂ O ₂		antioxidants, hypocholesterolemic(Siswadi et al.,2021)
6.	15.88	cis-11,12-Epoxytetradecen-1-ol	270.41	C ₁₆ H ₃₀ O ₃		-No activity
7.	16.98	8-octadecenoic acid methyl ester	296.4879	C ₁₉ H ₃₆ O ₂		antibacterial and antifungal properties (Belakhdar et al.,2015)
8.	19.62	1-tetradecanol, 2 decyl	354.7	C ₂₄ H ₅₀ O		anti-inflammatory (Taduri S et al.,2022)
9.	21.93	1 -hexocose	364.6911	C ₂₆ H ₅₂		antioxidant (Okaiyeto K et al.,2020)

Table 5.13 GAS CHROMATOGRAPHY–MASS SPECTROMETRY
INTERPRETATION

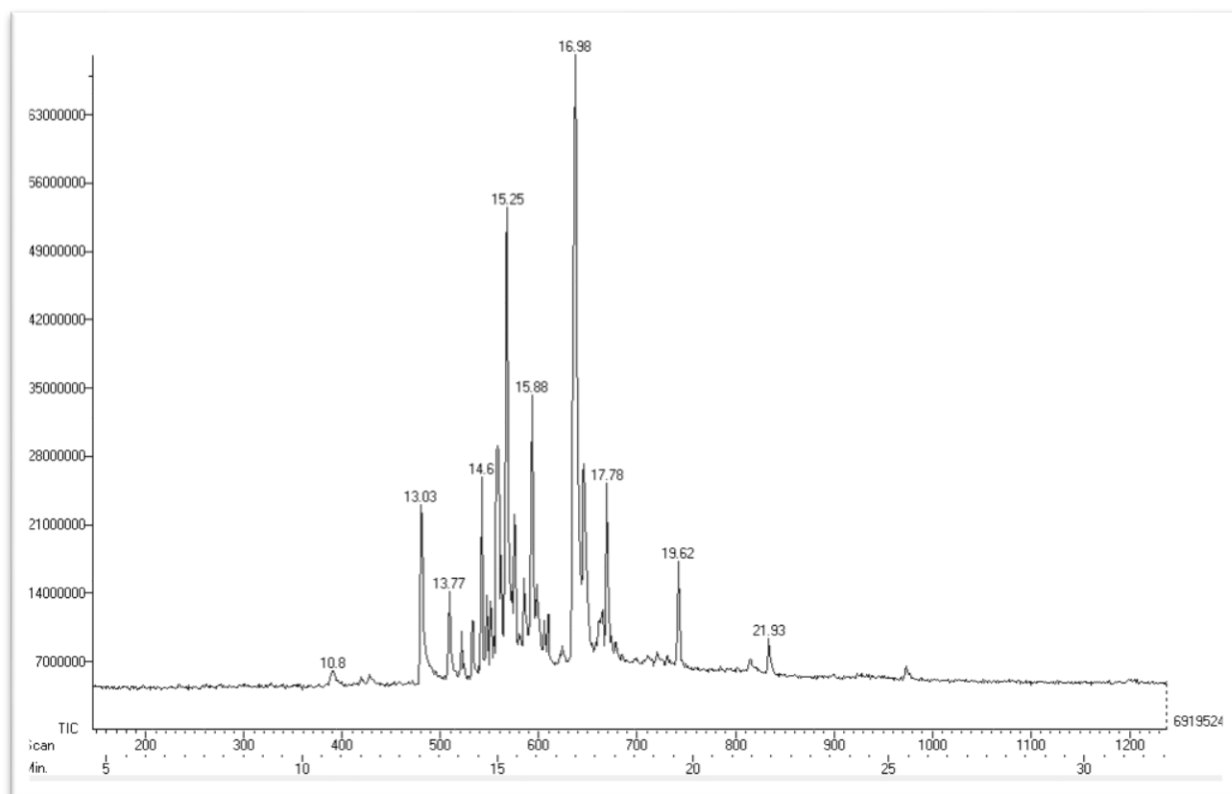


Fig 5.17 GAS CHROMATOGRAPHY–MASS SPECTROMETRY GRAPH

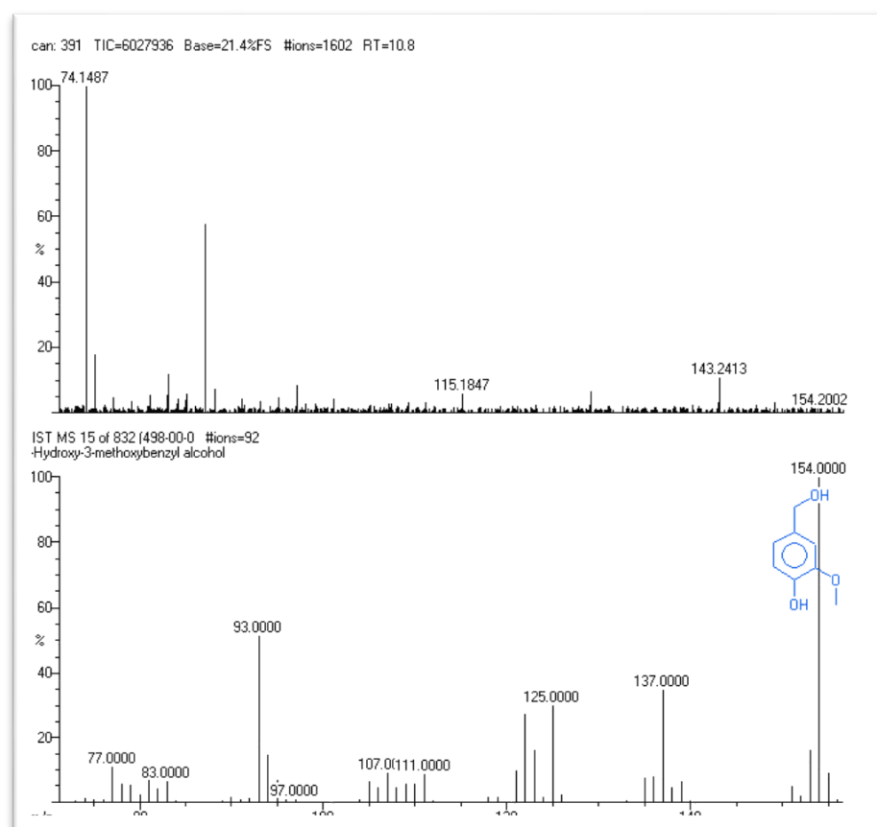


Fig 5.18 4-HYDROXY-3-METHOXYBENZYL ALCOHOL

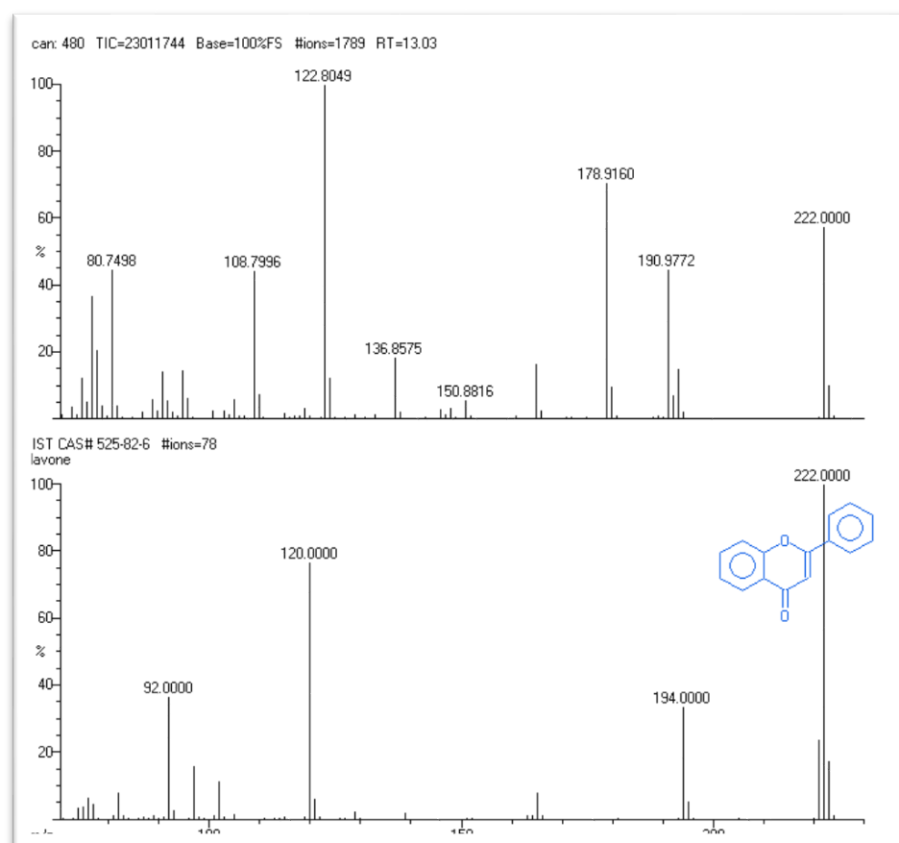


Fig 5.19 Flavone

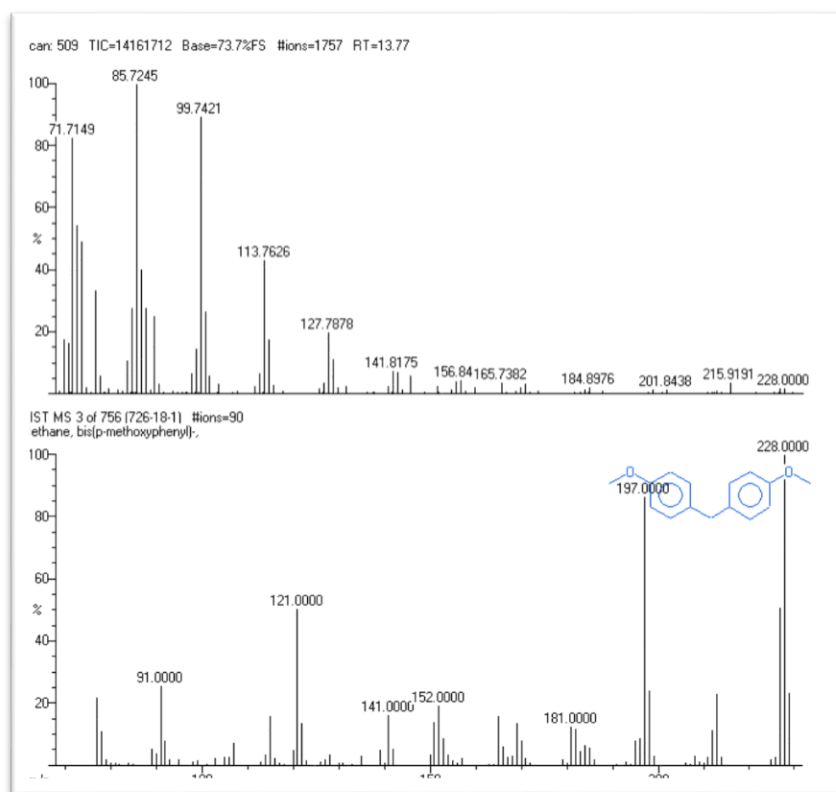


Fig 5.20 METHANE.BIS(p-methoxyphenyl)

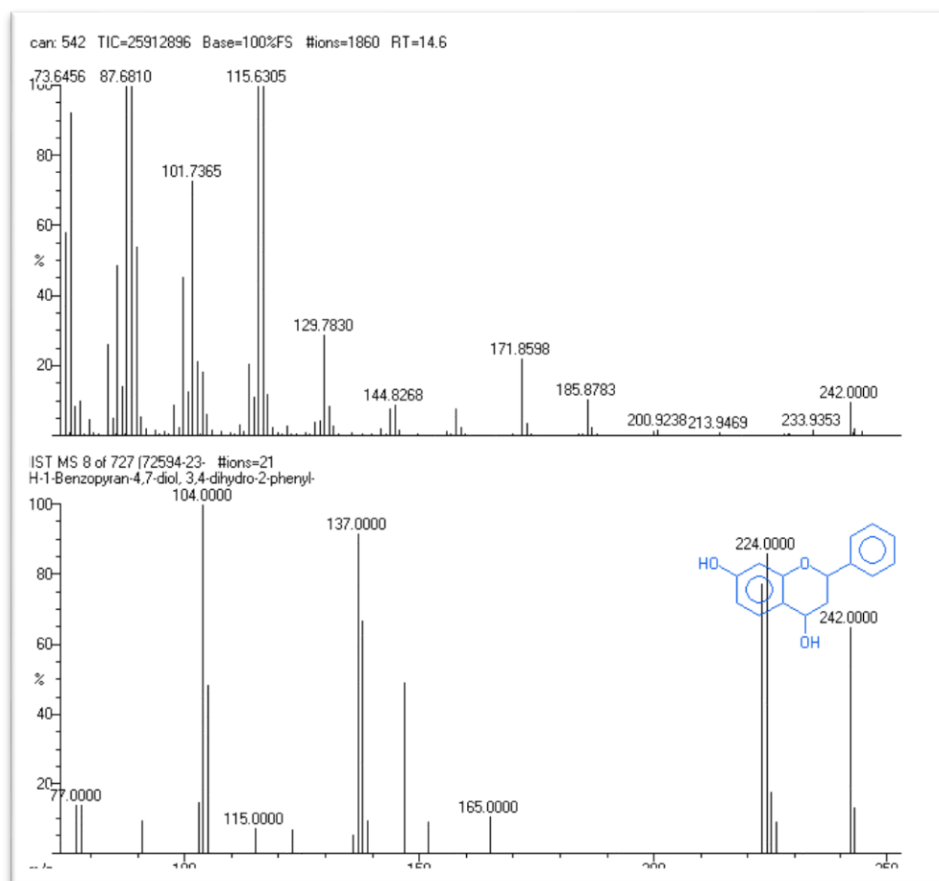


Fig 5.21 2H-1-benzopyran-4 7-diol,3,4-dihydro-2-phenyl

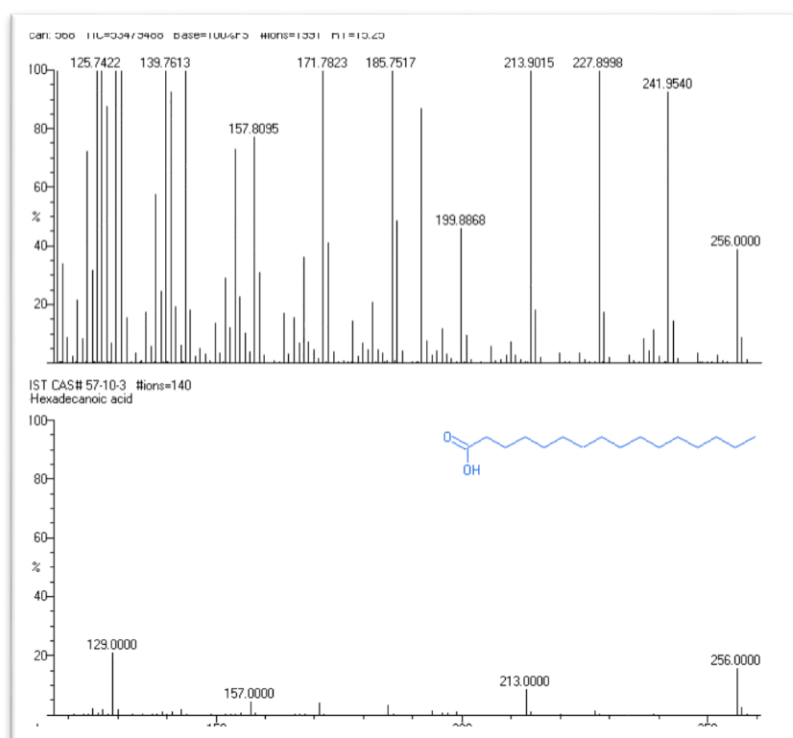


Fig 5.22 *n*-HEXADECANOIC ACID

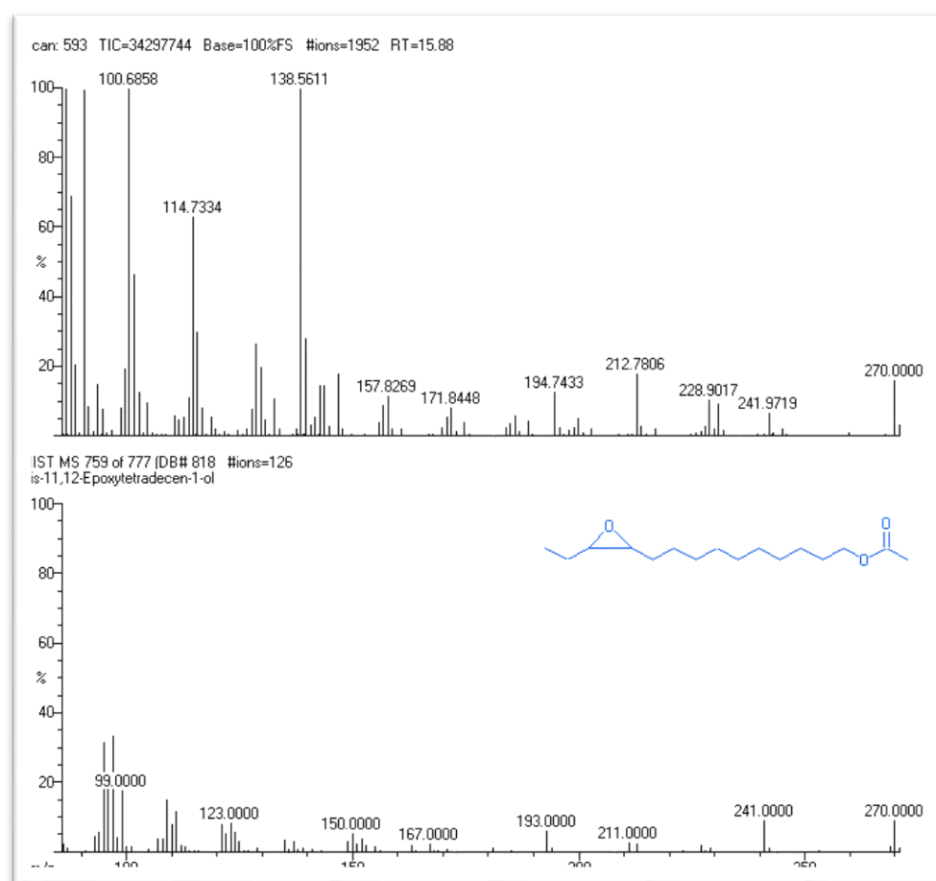


Fig 5.23 *cis*-11,12-Epoxytetradecen-1-ol

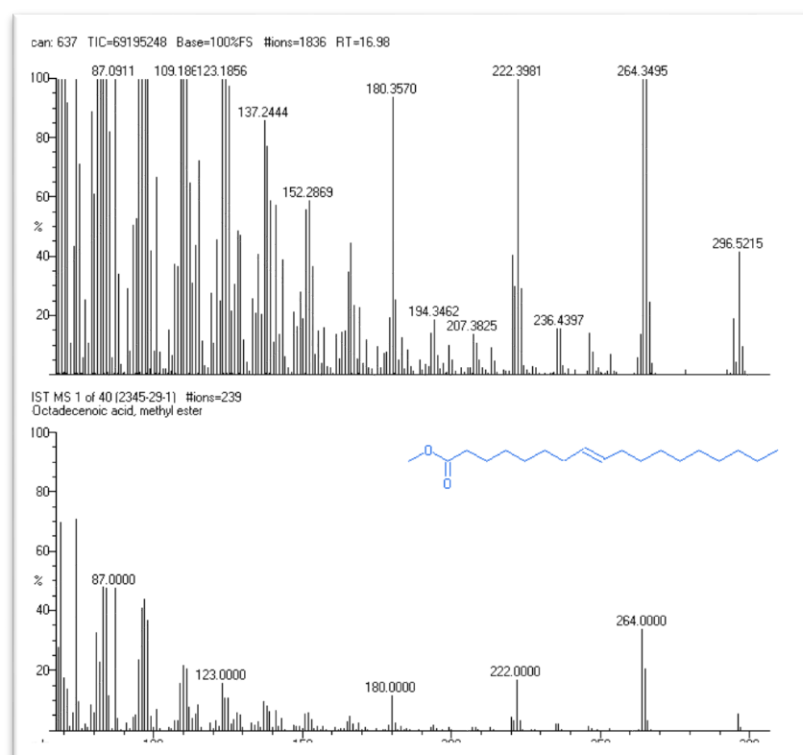


Fig 5.24 8-octadecenoic acid methyl ester

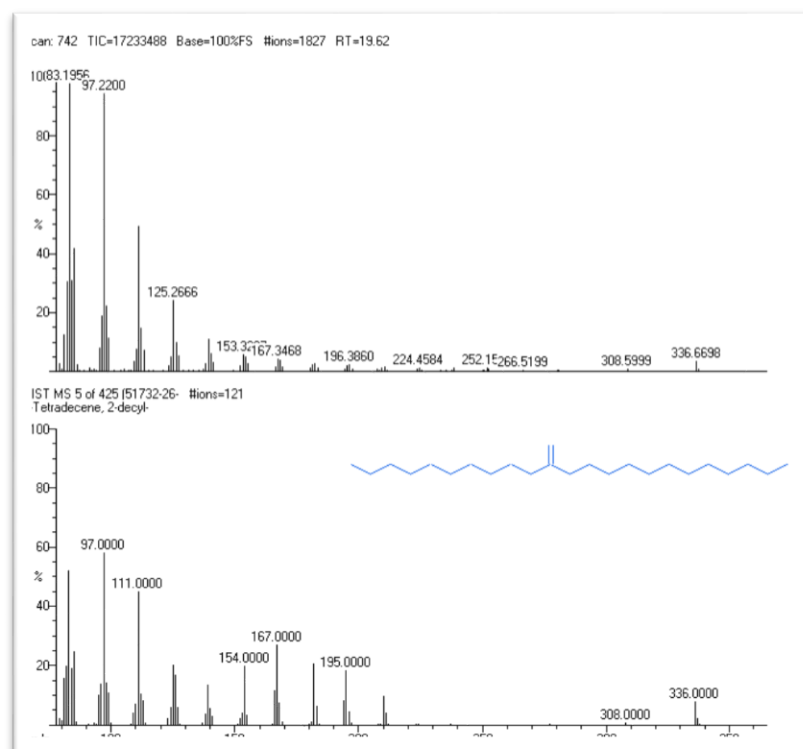


Fig 5.25 1-tetradecanol, 2 decyl

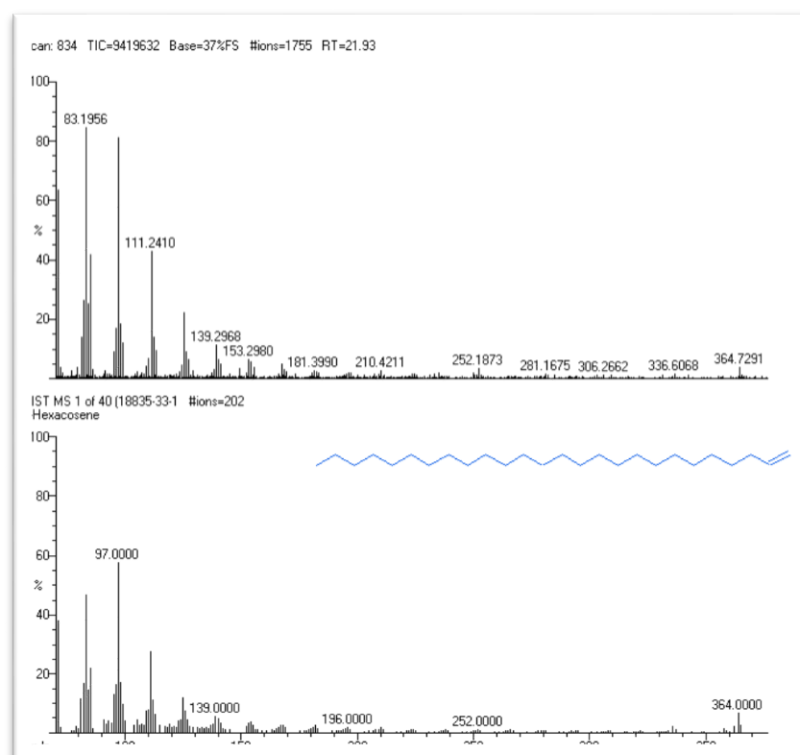


Fig 5.26 1-hexocosence

CHAPTER- 6

SUMMARY AND CONCLUSION

The organic feed incorporated with probiotics was slightly better than commercially available synthetic pellets. The carbohydrate and protein content of the organic feed was 31.3 g and 63 g respectively. Antioxidant activity of extract shows that increase inhibition at high concentration compared with positive control. Anti-inflammatory activity of extract shows that increase inhibition at high concentration compared with positive control. Anti-bacterial activity of fermented rice water inhibits the growth of *E. coli* and *S. aureus*. The formulated organic feed was cost-effective, chemical-free and was able to use industrial waste effectively and to overcome the disadvantages of existing commercial rodent food and supplements.

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