Executive Summary of the Ph.D. Thesis entitled

Development, Standardization and Evaluation of Herbal Formulation for Obesity

A Thesis submitted to

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By
Mr. Bhavik Chauhan

Under the Guidance of
Dr. Rajashree C. Mashru

Faculty of Pharmacy
The Maharaja Sayajirao University of Baroda
Vadodara 390001

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Executive Summary

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1. Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health leading to reduced life anticipation on health or increased health problems. Obesity is one of the leading preventable causes of death worldwide. An inactive life style plays a noteworthy role in obesity. Worldwide there has been a large shift towards less physically demanding work and currently at least 60% of the world's population gets insufficient exercise due to increased use of mechanized transportation. An average obesity reduces life expectancy by 6 to 7 years. Obesity is from lattin word obesitas, which means stout, fat or plump. The causes for obesity are High glycemic effect, genetic disorder, eating disorder, stressful mentality and insufficient sleep [(1).] Obesity is disorder which involves excessive body fat. This fat accumulates into the adipose tissues and other organs like liver, skeletal muscles. It is measured by Body mass index (BMI) which is used to differentiate person as underweight, overweight, normal or obese. BMI is ratio of person's weight in kilogram to the square of heights in meters. A BMI ≥25 kg/m^2 is defined the person is overweight and ≥30 kg/m^2 is obese. Obesity is a one of the major possibility factor for increasing health problems. It leads to hypercholestremia, hyperlipidemia, atherosclerosis, hypertension, diabetes mellitus etc.[(2)] The obesity can be categorised into two way .1) excessive intake of foods with high salt, fats and sugars but loss of minerals, vitamins and other nutrients. 2) Decreased or no exercises and other physical activity because of more sedentary life style and more use of transportation. Therefore the main etiology behind obesity is an imbalance between energy uptakes and expended. Our body needs some energy or calories from foods for basic functions. When calorie consumed and expended are equal then body weight is maintained. Because of highest mortality and morbidity, obesity requires proper management and treatment**.** [(3)]

This includes pharmacotherapy, diet plan and exercises. Certain foods that inverts metabolism of fats and lipids should be avoided. Statins like drugs, for example Atorvastatin inhibits HMG Co A reductase enzyme and widely used as allopathic treatment for obesity. Others are bile acid sequestrants, Fibrates, Niacin and Orlistat are the pharmacological treatment for obesity. But the major side effects associated with these drugs are rhabdomyolysis and others are allergic reactions such as wheezing, shortness of breathing, cough, swelling of face, tongue etc [(4)]. To overcome all these side effects herbal products are safe, having no or less side effects as compared to chemically synthesized compounds. Although herbal drugs are easily available without any prescription and advancement in

technology, herbal preparations are still in contact with pharma market due to their wider acceptance, cost effective and faith of people in using herbs that it is 100% natural origin means safe, thereby nowadays herbal products and their demand is going to be increased[(5)]. Present review focuses on mechanism, Pathophysiology behind the obesity and various herbs used in treatment of obesity.

1.1 Hyperlipidemia and Obesity:

Hyperlipidemia and obesity both are connecting condition. Hyperlipidemia is a condition in which there is elevation of plasma lipids in the blood, commonly LDL. It is also termed as hypercholesterolemia or hypelipoproteinemia. Increasing in lipids like LDL, triglyceride and cholesterol are mainly responsible. Hyperlipidemia can occur either due to over production or impaired removal of lipoproteins and defects in lipoprotein or its receptor. There are three types of lipoproteins –LDL, VLDL and HDL. Almost all the dietary fats are absorbed from the intestinal lumen into the intestinal lymph and packed into chylomicrons. These lipoproteins move into the blood stream where they got hydrolysed by endothelial lipoprotein lipase which hydrolyzes the triglyceride into glycerol and non-esterified fatty acids. After which the chylomicron remnants are absorbed in the liver and packaged with cholesterol, cholesteryl esters and ApoB100 to form VLDL. Low density lipoproteins in excessive quantities accelerate the deposition of LDL on artery walls and lead to atherosclerosis which is strongly related to ischemic heart disease (IHD) while as the high density lipoproteins prevent the deposition of LDL on artery walls and hence are preventive in nature [(9)]

1.2. Causes of Obesity

A. Energy Balance in the Development of Obesity

Obesity can result from a slight energy imbalance, which lead to a gradual but determined weight gain over a significant period. Some researchers have hypothesized that energy imbalance is the result of inborn metabolic characteristics; whereas others believe it is caused by poor eating and lifestyle habits, that is “gluttony and sloth. Positive energy balance occurs when energy intake is greater than energy expenditure and promotes weight gain. Conversely, negative energy balance promotes decrease in body fat stores and weight loss. Body weight is regulated by a series of physiological processes, which have the capacity to maintain weight within a relatively narrow range (stable weight). It is thought that the body exerts a stronger defence against under nutrition and weight loss than it does against over-consumption and

weight gain. Dietary intake and physical activity are important subsidizing factors in the development of obesity. If calorie intake is in excess of requirement it will be stored mainly as body fat. If the stored body fat is not utilised over time, it will lead to overweight or obesity. Inter-individual distinctions in energy intake, basal metabolic rate, unstructured physical activity, the relative rates of carbohydrate-to-fat oxidation, and the degree of insulin sensitivity seem to be closely involved in energy balance and in defining body weight in some individuals.

1.3 Remedies for obesity:

Pharmacological remedies for obesity:

Sr. no	Drug Class	Mechanism of Action	Examples	Side effects
1	HMG COA reductase enzyme inhibitor	Lowering total LDL inhibiting cholesterol biosynthesis	Atrovastatins, Fluvastatin Lovastatin, Simvastatin	Congestive cardiac failure
2	Fibrates	Enhancing activity of enzyme lipoprotein lipase	Gemfibrozil, fenofibrate	Upper GI disturbance, headache, myalgia
3	Nicotinic acid derivative	Inhibit lipolysis within adipocytes	Niacin	Hyperglycemia. increase uric acid
4	Bile acid sequestrants (Resin)	Bind with bile acid & promote bile acid excretion	Cholestipole, Cholestyramine	Abdominal fullness. constipation
5	Misc.	Inhibit free radicals	Omega 3 fatty acid, Probucol	

Prescription drugs for obesity:

Drug	Mechanism of Action	Side effects
Orlistat	Reduces fat absorption from the intestine by inhibiting pancreatic lipase and reduces triglyceride hydrolysis. Low fat diet is generally advised.	Steatorrhoea (oily stools).
Sibutramine	Centrally acting sympathomimetic amine that enhances satiety by inhibiting non- selective uptake of nor adrenaline, serotonin and dopamine	Hypertension, Serotonin syndrome
Metformin	It activates cAMP-activated protein kinase and suppresses hepatic gluconeogenesis activity.	Lactic acidosis, Gastro intestinal upset.
Rimonabant	It is an approved but infrequently used drug It is a	Severe depression and

	canabinoid CB1 receptor antagonist. It predisposes selectively acts on CB1 receptor in brain and peripheral organs. Reduces lipogenesis in liver.	neurodegenerative diseases Eg Alzheimer's disease.

Herbal Remedies for the obesity:

Sr.no	Anti-obesity function	Herbs
1	Inhibiting pancreatic lipase activity	Chitosan,green tea
2	Enhancing thermo genesis	Sea Weed, Bitter Orange, Soybean
3	Preventing adipocyte differentiation	Turmeric, Capsicum, Palm Oil, Banana Leaf, Brown Algae, Garlic, Flaxseed, Black soybean, Kokam fruit
4	Enhancing lipid metabolism	Herb Teas, Cinnamon, Guggul Lipid
5	Decreasing appetite	Pine Nut, Pomegranate Leaf,Ginseng, Hoodia Gordonii, Aghedo, Methi Seeds

1.4 WHO Guidelines for Quality Assessment of Herbal Medicines

World health organization has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use in toady. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. According to WHO, "Herbal Medicines" should be regarded as, "Finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substance of this nature [(9)]

A method of identification and quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g., “chromatographic fingerprint") to ensure consistent quality of the product[(10)]. Multicomponent botanical formulations can be standardized with newer techniques such as

high pressure thin layer chromatography (HPTLC), liquid chromatography and mass spectroscopy. The value of animal testing to establish safety and toxicity is also critical for the botanicals used in traditional forms prepared using drugs with a narrow therapeutic index. Nevertheless, all the critical pharmacopoeial tests such as dissolution time, microbial, pesticide and heavy metals contamination etc. must be in accordance with global standards and all the herbal medicines manufactured must be in accordance with current good manufacturing procedures for herbs.

1.5 Quality of herbal products:

The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good Agricultural Practices (GAP) can control this. These include seed selection, growth conditions, and use of fertilizers, harvesting, drying and storage. In fact, GAP procedures are, and will be, an integral part of quality control. Factors such as the use of fresh plants, age and part of plant collected, period, time and method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, and hence the therapeutic value of herbal medicines. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Using cultivated plants under controlled conditions instead of those collected from the wild can minimize most of these factors. Sometimes the active principles are destroyed by enzymatic processes that continue for long periods from collection to marketing, resulting in a variation of composition. Thus proper standardization and quality control of both the raw material and the herbal preparations should be conducted [11].

1.6 Standardization of Herbal Formulations

Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices; following a

recipe is not sufficient for a product to be called standardized. Therefore, the presence of the word "standardized" on a supplement label does not necessarily indicate product quality. When the active principles are unknown, marker substance(s) should be established for analytical purposes and standardization. Marker substances are chemically defined constituents of herbal drug that are important for the quality of the finished product. Single or multiple markers can be used to ensure that the concentration and ratio of components in an herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates, and in the final dosage forms. In this way, multiple markers or chromatographic fingerprints give information assisting manufacturing control and assuring batch-to-batch consistency [(12)].

1.7 Medicinal Plants used for the treatment of obesity:

A large number of herbal medicines and supplements are available in current market for the management of obesity. They all are having not same effects; reason behind this is the targets they focus are unique so all follow different mechanism of action. The basic principle behind antiobesity drugs are maintains the energy balance in the body that is equilibrium between energy intake and expenditure [(13)]. Following are the plants can be used for the treatment of obesity.

Table 1.1 List of the plant used in obesity

Botanical name	English name/Common name	Parts used	Reference
Acacia arabica	Babbula	Gum, bark, leaf, fruit-pods	14.
Achyranthus aspera	Apamarga	Root, seed, leaf, whole plant	15.
Aconitum heterophyllum	Ativisha	Root, rhizome	16.
Acorus calamus	Vacha	Rhizome	17.
Adathoda vasica	Vasa	Leaf, root, flower	18.
Allium sativum	Garlic	Stem, Fruit	19.
Aloe vera	Kumari	Leaf, root	20.
Betula utilis	Burja	Bark, nodes	21.
Camelia sinensis	Green Tea	Leaves	22.
Catharuths roseus	Barmasi	Whole plant	23.
Coriander sativum	Coriander	Fruits	24.
Cassia tora	Chakramardha	Seed, leaf, root	25.
Cedrus deodara	Devadaru	Hearwood oil	26.
Embelia ribes	Vidanga	Fruit	27.
Emblica officinalis	Amalaki	Fruit	28.

Garcinia indica	Vrikshamla	Fruit, root, bark, oil	29.
Gymnema sylvestre	Meshashringi	Leaf, root, seed	30.
Holarrhena antidysentrica	Kutaja	Seed, bark	31.
Momordica charantia	Karavellaka	Fruit, whole plant, leaf, root	32.
Moringa oleifera	Sigru	Root, bark, seed	33.
Picrorhiza kurroa	Katuka	Root	34.
Piper longum	Pippali	Fruit, root	35.
Piper nigrum	Maricha	Fruit	36.
Plumbago zeylanica	Chitraka	Root, bark	37.
Punica granatum	Pomegranate	Fruit rind ,leaves	38.
Terminalia arjuna	Arjuna	Bark, root, leaf	39.
Terminalia bellerica	Bibhitaka	fruit	40.
Terminalia chebula	Haritaki	fruit	41.
Terminalia tomentosa	Asana	Bark, heartwood	42.
Thea sinensis	*Oolong tea*	Leaf	43.
Tinospora cordifolia	Guduchi	Stem, root	44.
Trachyspermum ammi	Yavani	Fruit	45.
Tribulus terrestris	Gokshura	Fruit, root, whole plant	46.
Trigonella foenum graceum	Methika	Seed, leaf, whole plant	47.
Valeriana jatamansi	Tagara	Root	48.
Zingiber officinale	Shunti	Rhizome	49.

1.8 *In silico* Approach:

An *insilico* study is one executed via simulation on a computer. In silico simulations are frequently used to expect how a compound will react with proteins in the body or with pathogens.

Common applications for *in silico* studies include:

Drug candidate screening (molecular docking studies), Prediction of adverse drug reactions, Whole cell simulations and Sequencing (in silico PCR)

In silico models are computational simulations of a complex system in the form of comparisons or rules. In silico computational models provide the tools to qualitatively and quantitatively evaluate many treatments on specific diseases and to test a larger set of different conditions (e.g. dosing). These models are abstract representations used to model human diseases, a concept which is often limited by in-vitro/vivo techniques.

These types of models are becoming gradually popular within the pharmaceutical industry drug development especially. Computer-aided drug design (CADD), for example, is a group of in silico methods which deal a cost-effective way of finding drug candidates. The ligand-based design of CADD uses reference structures collected from the compounds known to interact with the target, and analyses their 2D/3D structure. [48]

Alternative of available biological evaluation methodology:

Computer-based models and tactics, like *in silico,* have the potential to lessen the number of animals needed in a study and maybe one day, replace them entirely. The shift from animal models to computational versions has been a focus for the pharma industry for a number an important reasons.

- While animal models remain to show great value in preclinical studies, concern for the mental and physical well-being has seen a number of companies spread in alternative ways of modeling disease in order to reduce the number of animals used within drug development.
- The husbandry complicated with animals in research can be extensive, requiring large, expensive buildings for their maintenance with resources like food and frequent care required. In addition, some animal models can take a long time to progress which slows down the drug development process and again increases cost. Epilepsy rodent models, for example, can sometimes take up to a year to develop pathological variations in the brain before experiments can begin.

2. Research Methodology

2.1 Literature Review:

As we know there are so many marketed formulations in allopathic system available for the treatment for obesity but us also aware that they are having several uncontrolled side effects. They are having different targets to treat the obesity. There are some class of the drugs mention here which are used.

Sr.no	Medicines	Side effects
1	HMG-CoA Reductase Inhibitors - Statins ex. Atorvastatin,	Myalgias and Rhabdomyolysis.
2	Bile acids binding Resins	Constipation, nausea, and May increase triglycerides;
3	Ezetimibe	Arthralgias and myalgias more common when combined with a statins
4	Niacin	Flushing is common; may be reduced with aspirin pre treatment May increase uric acid and glucose levels.
5	Fibrates	Gastrointestinal upset, rash, and abdominal pain are common. Decreased renal function and myopathies are rare. Increases risk of gallstones by 1 to 2 per cent

2.2 Current Herbal approach for the obesity:

Herbal plants for weight reduction may be effective in the treatment of obesity and associated disorders. Consistent and safe herbal product for weight reduction is a need of developed and developing countries. In our literature survey, herbal plants showed potential effects on weight control. A variety of natural products, including crude extracts and isolated compounds from plants, can induce lipid profile reduction and prevent obesity. Therefore, they have been widely used in treating obesity. The botanical drugs can be developed faster and cheaper than conventional single-entity pharmaceuticals. Botanicals are safe, natural, and cost effective alternatives to synthetic drugs.

Sr.no.	Formulation	Content	Dose/Duration
1	Ayurslim	*Garcinia cambogia*, *Balsamodendron mukul*, *Gymnema sylvestre*, *Terminalia chebula*, *Trigonella foenum-graecum*	2 capsules, twice daily for 6 months
2	Forslean	250 mg of a standardized extract of *Coleus forskohlii(*forskolin 25 mg*)*	1 capsule in the morning and 1 in the evening, 30 minutes before a meal. For 4 - 8 weeks
3	Lipotrim	Chromium 100 mcg, *Garcinia cambogia* fruit extract 50 mg.	NA
4	Slimex	Aqueous extract of *Hordeum vulgare*, *Polygonatum multiflorum*, *Dimocarpus longan*, *Ligusticum sinense*, *Lilium brownii* and *Zingiber officinallis*	Six week period
5	Slim339	Proprietary blend of *Garcinia cambogia* extract with calcium pantothenate (standardized for the content of hydroxycitric acid and pantothenic acid) and extracts of *Matricaria chamomilla*, *Rosa damascena*, *Lavandula officinalis* and *Cananga odorata*	1 tablet of Slim339 three times per day (60 - 90 min before a meal), for 60 days

2.3 Recent literature for Drug targets of obesity:

A large number of herbal medicines and supplements are available in current market for the managing of obesity. They all are having not same effects; reason behind this is the targets they emphasis are unique so all follow different mechanism of action. The basic principle behind ant obesity drugs are maintains the energy balance in the body that is symmetry between energy intake and expenditure [(1)]. The main methodologies follow either effect of these drugs on nervous system or effect of supplement on physiological function. All targets by which antiobesity drugs or supplements acting are described as below:

2.3.1 Pancreatic lipase enzyme inhibition:

Dietary fats are absorbed in the intestine by action through Lipase enzyme that converts fats in to the free fatty acids and monoglycerides. If pancreatic lipase enzyme is inhibited then finally the formation of fatty acid is blocked which in turn leading to weight loss. This inhibitory activity possesses phytoconstituents which includes saponins, phenolic compounds and flavanoids and caffeine. Pharmacological agent Orlistat works on this target [(2)].

2.3.2 Thermogenesis:

Increases thermogenesis by metabolism from generation of ATPs thereby conversion of food energy as heat and it finally leads to weight loss. There are three types of adipose tissue: White, brown and beige adipose tissue. In thermogenesis, brown adipose tissue plays important role in obesity by dissolving excess energy as heat and thereby governing energy balance. Various naturally occurring compounds like caffeine, capsaicine are used in treatment for obesity [(3)].

2.3.3 Lipid Metabolism:

The pharmacological target for lipolysis can be predicted by stimulating triglyceride hydrolysis in order to lower down to the fat stores. This will need oxidation of newly released fatty acid, some examples are the flavanoids of leaves of the plant Nelumbo mucifera activate the B-adrenergic receptor and though this pathway it leads to overturn the body weight gain. The another example is caffeine, a major phytoconstituent found in oolong tea acts by binding with the phospholipid phosphate group and interface between the lipase and triglyceride portion of lipid droplets and thereby boosts lipolysis [(4)].

2.3.4 Centrally acting mechanism:

Body weight can be sustained by intake of foods. Many drugs act directly on their effect on the receptor within the central nervous system. The status of body stores and adiposity is controlled by three main hormones leptin, insulin and gastrointestinal peptide such as ghrelin and they all interconnect to the central nervous system. For example green tea extract has reported to normalize the plasma leptin concentration. A number of natural appetite suppressants herbs reduce the expression of hypothalamic neuropeptide Y or serum leptin levels. The other mechanism is to alter the various hypothalamic neuropeptide's CNS level and key CNS appetite neurotransmitter's level via peripheral satiety peptide system and

thereby supress the appetite (5). The phytoconstituents found in green tea like catechin-epicatechin, epigallocatechin stimulate thermogenesis by inhibition of catechol- O-methyltransferase enzyme that is liable for degradation of norepinephrine. The naturally occurring Hydroxy citric acid obtained from *Garcenia cambogia* is a potential appetite suppressant. It inhibits adenosine 5- triphosphate-citrate lyase which stops acetyl co enzyme-A production and decreases fatty acid synthesis.

2.4. Selection of herbs for formulation:

Many medicinal plants are used in the treatment of the obesity. A variety of natural products, including crude extracts and isolated compounds from plants, can induce lipid profile reduction and prevent obesity. Therefore, they have been widely used in treating obesity. The botanical drugs can be developed faster and cheaper than conventional single-entity pharmaceuticals. Botanicals are safe, natural, and cost effective alternatives to synthetic drugs. Different plants having different mechanism to treat the obesity based on the presence of the active metabolites like flavonoids, alkaloids, glycosides and phenolics.
From the extensive literature review following herbs are selected for the formulation

- ✓ *Garcenia indica (Fruit)*
- ✓ *Murraya koenigii (Leaves)*
- ✓ *Commiphora mukul* (Gum)
- ✓ *Achyranthes aspera* (Seed)

2.5 Herbal Tablet formulation:

A tablet is a unit pharmaceutical dosage form. Tablets may be defined as the solid unit dosage form of medicament or medicaments with or without suitable excipients and prepared either by moulding or by compression. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted from a powder into a solid dose. In Ayurveda many Medicinal Plants used as Anti-obesity agents but they are providing it as in the form of Powders. Most of the herbs from the natural source are moisture sensitive, volatile, and hygroscopic so, the powder shows the moisture sensitivity and stability issues. To overcome this common problem we thought of the herbal tablet using the suitable pharmaceutical excipients. This will help to increase the rate of acceptance and uniformity of

dose and stability of the herbal actives. Following are some of the methods which are used for the tablet formulation [51].

2.5.1 Methods Used for tablet formulation:

Table. Methods of tablet praparation

Methods	**Applicability**	**Comments**
Direct compression	Low and intermediate drug contents (usually 2-30%)	Not suitable for very low or high drug contents. Susceptible to variations in drug substance properties.
Dry granulation	Low to high drug content depending on drug substance properties	Avoids the use of water. Some limitations on utility dependent on the properties of the drug substance.
Wet granulation	All drug contents (from very low to very high).	Flexible but resource intensive process which is hard to automate. Usually uses water in the manufacturing process so may not be suitable for moisture sensitive products

2.5.2 Excipients used for tablet formulation:

The excipients used in tablet compression are as follows:

Class	**Excipients**	**Concentration**
Direct compressible diluents	MCC Ethyl Acetate Dicalcium Phosphate Lactose Sucrose Avicel PH 102	5-15% 1-5% 5-20% 2-25% 5-20% 2-10%
Super disintegrants	Starch AC-Di-sol(Na-CMC) Sodium Starch Glycocollate Cross carmellose sodium Crosspovidone	10-15% 0.5-10% 2-5% 0.5-5%
Lubricants	Talc Magnesium stearate	1%
Preservatives	Methyl paraben Propyl paraben	1% 0.2%
Adsorbant	Syloid	0.1- 2%

Table. Excipients used for tablet

2.5.3 Evaluation Parameters of Tablets

- ✓ Thickness and diameter
- ✓ Weight variation
- ✓ Hardness
- ✓ Friability
- ✓ Drug content

- ✓ Disintegration time
- ✓ In-vitro dissolution

For Polyherbal tablet, dissolution test is not required. Disintegration test is required for uncoated tablets and limits are as follows:

	Medium	Temperature	Time
According to USP	Simulated gastric fluid	37'C	Not exceed 30 min
According to BP	Water	37'C	Not exceed 15 min

2.6 Analytical Method Development and Validation:

2.6.1 Analytical Method development:

Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation products and related substances, residual solvents as well as herbal preparations etc.

- ✓ It has become an integral part of the requirements of the regulatory organization.
- ✓ Analytical method development finally results in official test methods.
- ✓ These methods are used in quality control laboratories to ensure the identity, purity, safety, efficacy and performance of drug products.
- ✓ Regulatory authorities are placing greater emphasis on analytical methods in manufacturing.

Criteria for the Development of New Analytical Method

The reasons for the development of newer methods of drugs analysis are -

- ✓ The new drug or drug combination may not be official in any pharmacopoeias. A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- ✓ Analytical methods may not be available for the drug in the form of formulation excipients.
- ✓ Analytical methods for a drug in combination with other drugs may not be available.
- ✓ Analytical methods for the quantitation of the drug in biological fluids may not be available.
- ✓ The existing analytical procedures may require expensive reagents and solvents.

- ✓ It may also involve cumbersome extraction and separation procedure and these may not be reliable.

2.6.2 Validation of Analytical Method:

According to USP General chapter <1225> "Validation is the process of providing documented evidence that the method does what it is intended to do" In other words the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use

Advantages of analytical method validation:

- ✓ The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.
- ✓ Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.
- ✓ Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

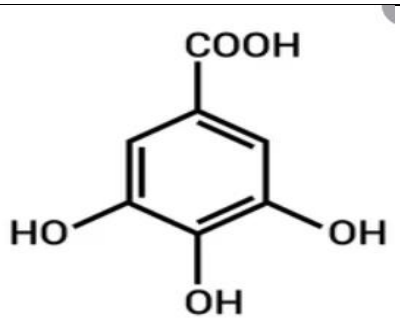
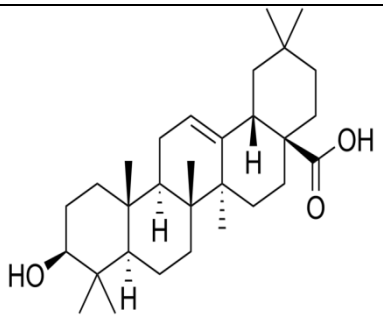
Validation Parameters

- ✓ Specificity
- ✓ Linearity & Range
- ✓ Precision
    - o Method precision (Repeatability)
    - o Intermediate precision (Ruggedness)
- ✓ Accuracy (Recovery)
- ✓ Solution stability
- ✓ Limit of Detection (LOD)
- ✓ Limit of Quantification (LOQ)
- ✓ Robustness.

2.7 Selection of Markers for AMD by HPLC and HPTLC:

Both phytoconstituents Gallic acid and Oleanolic acid are analysed simultaneously by Reversed Phase HPLC and basic requirement for these are as follows:

Table Markers for HPLC

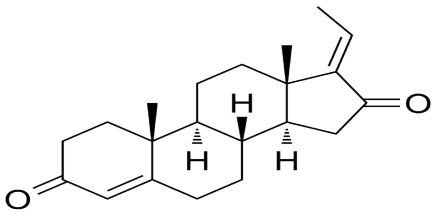
GALLIC ACID	OLEANOLIC ACID
	
Solubility: alcohol, ether, glycerol, acetone; negligible in benzene, chloroform, petroleum ether	In Methanol
Molecular weight: 170.12gm/mol	456.7 gm/ mol
Formula: C7H6O5	C30H48O3
Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, found in gallnuts, sumac, tea leaves, oak bark, and other plants.	Oleanolic acid or oleanic acid is a naturally occurring pentacyclic triterpenoid related to betulinic acid. It is widely distributed in food and plants where it exists as a free acid or as an aglycone of triterpenoid saponins.

HPTLC:

Simultaneous estimation of *Gallic acid*, *Oleanolic acid* and *E-Guggulsterone* are developed and for that basic analytical information are as follows:

E-guggulsterone:

Molecular formula: C21H28O2
Molecular weight: 312.453 gm/mol
Slightly soluble in Methanol, DMSO and Chloroform



2.8 *In Silico* Method for screening of marker compounds for obesity

Over the past decades, many obesity drugs have been expelled due to serious side effects. this is why numerous studies are attentive on traditional medicine, which has remained Wide spread in all regions of the emerging world due to its powerful pharmacological activities, fewer side effects, and quite low cost. In significance, we have tried in this research to

confirm the experimental results of plants known in the literature by computational chemistry techniques. [54]

The OB-receptor or leptin receptor (LR) is crucial for energy homeostasis and regulation of food uptake. Leptin is a 16 kDa hormone that is mainly secreted by fat cells into the bloodstream. Under normal circumstances, circulating leptin levels are proportionate to the fat body mass. Sensing of elevated leptin levels by the hypothalamic neuro-circuitry activates a negative feedback loop resulting in reduced food intake and increased energy expenditure.

In this study, we done molecular docking to identify therapeutic agents against obesity, and it is a technique that makes it possible to predict or study interactions that are the main factors having an important impact on the affinity of a ligand for a receptor. In addition, this study helps us to intend some plants as a treatment for weight loss.

Protein Selection:

Nine protein molecules were designated that are involved in the pathogenesis of obesity. The selected proteins, i.e., suppressor of cytokine signaling 3(Socs3), cholesteryl ester transfer protein (CETP), C-Jun N-terminal kinases-1(JNK1), lamin A/C, peroxisome proliferator-activated receptor γ (PPAR-γ), adiponectin, α-amylase, aldose reductase, and α-glucosidase were retrieved from Research Collaboratory for Structural Bioinformatics (https://www.rcsb.org/) protein data bank. These modified proteins were then saved in the.pdb format.

3. Key Finding

3.1 Proximate Analysis of Each Plant Extracts:

The plant extracts were subjected to various qualitative tests to determine presence of various phytochemical classes.

Chemical Class	Test name	Aghedo extract	Garcincia ext	Curry leaves extract	Guggul extract
Carbohydrate	**Molisch's test**	-	+	+	-
Proteins	**Biuret test**	-	+	+	+
	Millon's test	-	-	+	+
	Precipitation	-	-	+	+
	test				
Amino acids	**Ninhydrin test**	-	+	+	+
Steroids	**Salkowaski reaction**	-	-	+	+
	Libermann – Burchard reaction	-	-	+	+
Cardiac glycosides	**Legal's test**	-	-	-	+
	Libermann's test	-	-	-	+
Saponin Glycosides	**Foam test**	+	+	-	-
Flavanoids	**Fluorescence test**	-	+	+	+
Alkaloids	**Dragendorff's test**	+	-		
	Mayer's test	+	-	-	-
	Hager's test	+	-	-	+-
	Wagner's test	+	-	-	+
Tannins and Phenolics	**Ferric chloride test**	-	+	+	-
	Lead acetate test	-	+	+	-

3.2 Thin Layer Chromatography of each Plant Extracts:

Thin Layer chromatography is effective standardization technique through which the presence of marker compound is determined in relevant plant extracts.

Extracts	Mobile Phase	Spraying reagent And detection wavelength
Aghedo extract	Toluene:EthylAcetate:Methanol: Acetone(14:4:1:1)	Methanolic sulfuric acid 320 nm
Garcinia extract	n butanol: Formicacid: water (4:2:4)	UV at 273 nm
CurryLeaves Extract	Pet.Ether:Chloroform(7:3)	UV at 254 nm
Guggul extract	Toluene: Acetone(9:1)	UV at 240 nm

3.3 Estimation of Secondary metabolites:

3.3.1 Determination of Total Phenolic content:

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content.

Results for total phenolic content:

Plant Extract	Total phenolic content (w/w)
Aghedo	0.77%
Garcinia	0.59%
Curry leaves	5.197%
Guggul	4.03%

3.3.2 Determination of Total flavanoid content:

Total flavonoid content was measured by the aluminium chloride colorimetric assay.

Result of total Flavanoid content:

Table Total Flavanoid Content

Plant extract	Total Flavanoid content (w/w)
Aghedo	0.37%
Garcinia	0.67%
Curry leaves	4.816%
Guggul	6.055%

3.4 Formulation and Development:

Chemical and Reagents: Avicel PH 102, Talc, Magnesium Stearate, Sodium Starch Glycocollate, Cross Carmellose sodium, Syloid.

Instruments used: Tablet Compression machine, weighing balance etc.

3.4.1 Concentration of plant extract

Table Active concentration

Sr.No.	Plant Extract	Range of dose
1	*Achyranthus aspera*	75-150mg
2	*Garcinia Indica*	50-200 mg
3	*Murraya koenigii*	125-500 mg
4	*Commiphora mukul*	100-300 mg

3.4.2 Optimization of excipients was done by using response surface D optimal Factorial design

3.4.2.1 Variables for response

Table 2Response variables

Sr. No.	Variables	Lower limit	Upper limit	Unit
1.	Avicel PH102	10	20	gm
2.	Sodium Starch Glycocollate	0.5	10	gm

3.	Cross Carmellose sodium	0.5	5	gm
4	Syloid	0.25	5	gm

3.4.2.2 The obtained design matrix

Table Design Matrix

Sr. No.	Factor 1(Avicel PH 102)	Factor 2(S.S.G)	Factor 3(C.C.S.)	Factor 4 (Syloid)
1.	10	0.5	0.5	5.0
2.	14	6.64	0.57	0.25
3.	10	8.90	0.5	0.25
4.	20	10	0.5	5.0
5.	10	0.5	5.0	3.0
6.	20	5.03	2.86	0.25
7.	14	2.91	2.19	2.00
8.	10	0.5	0.5	0.25
9.	16.0	7.5	5.0	0.00
10.	20	10	0.5	0.25
11.	16	5.01	5.0	0.25
12.	20	0.5	0.5	0.50
13	11	0.5	4.32	3.0
14	10	10	5.0	0.50
15	10	10	5.0	0.5
16	20	10	5.0	2.00
17	16	7.5	5.0	3.00

3.4.2.3 Desired values of variables:

Table 3Desired values of variables

Sr.no	Variable	Goals
1.	Avicel PH102	Target
2.	Sodium Starch Glycocollate	Maximize
3.	Cross Carmellose sodium	Maximize
4	Syloid	Maximize
5	Disintegration time(Minute)	Minimize
6	Hardness (Kg/cm^2)	Maximize

3.4.2.4 Results of Responses:

	Std	Run	Block	Factor 1 A:Avicel PH102 gm	Factor 2 B:S.S.G gm	Factor 3 C:C.C.S. gm	Factor 4 D:Syloid gm	Response 1 Disintegration t Minutes	Response 2 Hardness Kg/cm2
	2	1	Block 1	10.00	0.50	0.50	5.00	16	2.9
	17	2	Block 1	14.10	6.64	0.57	0.25	15	3.5
	14	3	Block 1	10.00	8.93	0.50	0.25	11	2.1
	3	4	Block 1	20.00	10.00	0.50	5.00	15	4.9
	1	5	Block 1	10.00	0.50	5.00	3.00	14.5	2.6
	10	6	Block 1	20.00	5.03	2.86	0.25	19.5	5.6
	16	7	Block 1	14.05	2.91	2.19	2.00	17	3.9
	5	8	Block 1	10.00	0.50	0.50	0.25	17	3.1
	8	9	Block 1	16.00	7.50	5.00	0.00	13	3.5
	13	10	Block 1	20.00	10.00	0.50	0.25	17	4.2
	7	11	Block 1	16.00	5.01	5.00	0.25	15.5	3.8
	9	12	Block 1	20.00	0.50	0.50	0.50	25	6.2
	11	13	Block 1	11.00	0.50	4.32	3.00	16	5.2
	6	14	Block 1	10.00	10.00	5.00	0.50	9	2
	4	15	Block 1	10.00	10.00	5.00	0.50	6.5	1.8
	12	16	Block 1	20.00	10.00	5.00	2.00	13	3.4
	15	17	Block 1	16.00	7.50	5.00	3.00	10.5	4

Figure Result of responses

3.4.2.5 ANOVA Analysis for Disintegration time:

To identify the significant parameters and their interaction, analysis of variance was performed for each parameter. The values of the coefficients of A, B,C and D are related to the effect of these variables on the response. Larger coefficient means the independent variable has more potent influence on the responses.

Response 1 Disintegration Transform: None

*** WARNING: The Cubic Model is Aliased! ***

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	3691.19	1	3691.19			
Linear vs Mean	276.56	4	69.14	87.37	< 0.0001	Suggested
2FI vs Linear	4.93	6	0.82	1.08	0.4649	
Quadratic vs 2FI	1.20	4	0.30	0.18	0.9310	
Cubic vs Quadra	0.24	1	0.24	0.078	0.8264	Aliased
Residual	3.13	1	3.13			
Total	3977.25	17	233.96			

Sequential Model Sum of Squares [Type I]: Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Response 1 Disintegration time

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	276.56	4	69.14	87.37	< 0.0001	significant
A-Avicel PH102	*110.48*	*1*	*110.48*	*139.61*	*< 0.0001*	
B-S.S.G	*149.53*	*1*	*149.53*	*188.97*	*< 0.0001*	
C-C.C.S.	*25.43*	*1*	*25.43*	*32.14*	*0.0001*	
D-Syloid	*7.31*	*1*	*7.31*	*9.24*	*0.0103*	
Residual	9.50	12	0.79			
Lack of Fit	*6.37*	*11*	*0.58*	*0.19*	*0.9596*	*not significant*
Pure Error	*3.13*	*1*	*3.13*			
Cor Total	286.06	16				

Figure ANOVA for Response surface Linear Model

The Model F-value of 87.37 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, D are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

ANOVA study Results for Disintegration time

Std. Dev.	0.89	R-Squared	0.9668
Mean	14.74	Adj R-Squared	0.9557
C.V. %	6.04	Pred R-Squared	0.9355
PRESS	18.44	Adeq Precision	35.772

Figure 4 ANOVA study Results for Disintegration time

The "Pred R-Squared" of 0.9355 is in reasonable agreement with the "Adj R-Squared" of 0.9557. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 35.772 indicates an adequate signal. This model can be used to navigate the design space.Final Equation in Terms of Coded Factors:

Disintegration time = +14.97 +3.27* A -3.99* B -1.37* C -0.97* D

From the above equation we can observe that all the factors affected disintegration time to some extent. Decrease in concentration of Avicel PH 102 and increase in concentration of S.S.G leads to have a decrease in Disintegration time.

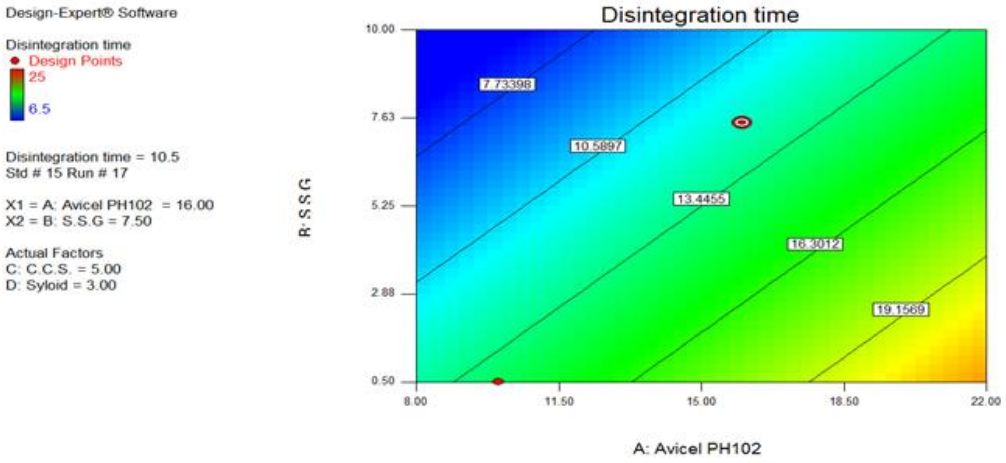


Figure Desirability plot contour for Disintegration time.

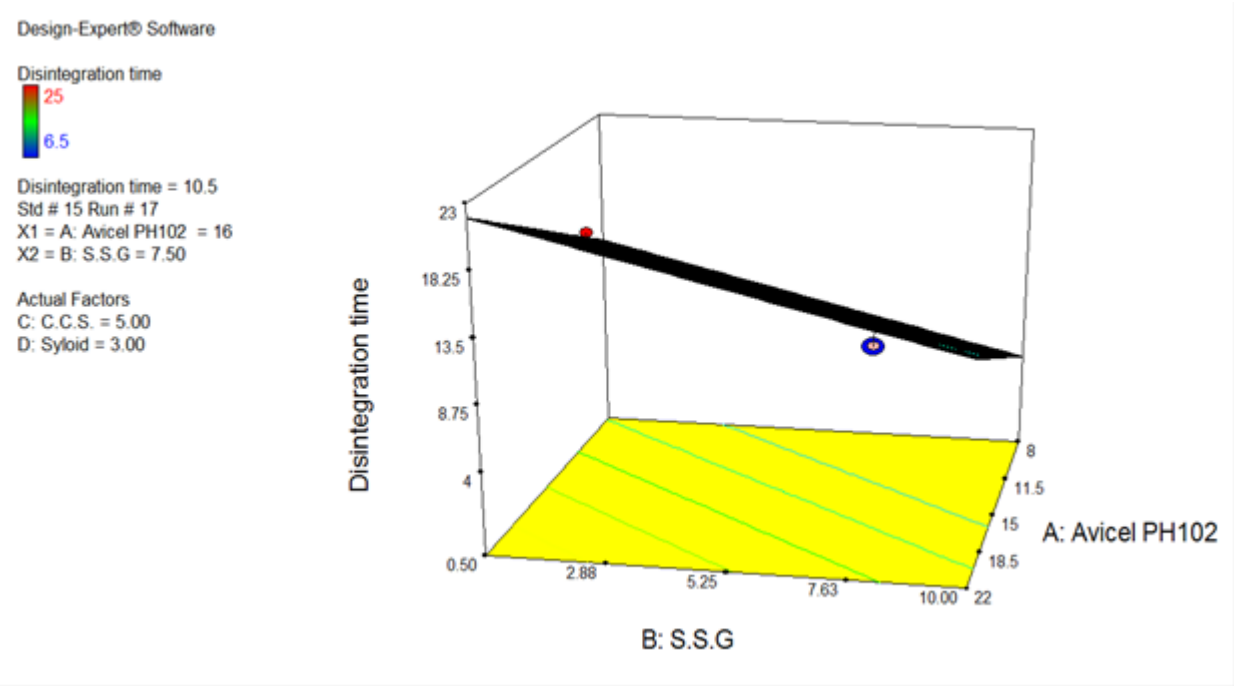


Figure Desirability plot (3D surface) for Disintegration time

3.4.2.6 ANOVA analysis for Hardness:

To identify the significant parameters and their interaction, analysis of variance was performed for each parameter. The values of the coefficients of A, B and C are relatedto the effect of these variables on the response. Larger coefficient means the independent variable has more potent influence on the responses.

ANOVA for Response surface Linear Model

Response 2 Hardness

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	20.29	4	5.07	11.97	0.0004	significant
A-Avicel PH102	*16.95*	*1*	*16.95*	*39.98*	*< 0.0001*	
B-S.S.G	*6.20*	*1*	*6.20*	*14.63*	*0.0024*	
C-C.C.S.	*0.038*	*1*	*0.038*	*0.091*	*0.7684*	
D-Syloid	*0.028*	*1*	*0.028*	*0.067*	*0.8006*	
Residual	5.09	12	0.42			
Lack of Fit	*5.07*	*11*	*0.46*	*23.03*	*0.1613*	*not significant*
Pure Error	*0.020*	*1*	*0.020*			
Cor Total	25.38	16				

Figure ANOVA for Response surface Linear Model

The Model F-value of 11.97 implies the model is significant. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

ANOVA study Results for Hardness:

Std. Dev.	0.65	R-Squared	0.7996
Mean	3.69	Adj R-Squared	0.7328
C.V. %	17.65	Pred R-Squared	0.5706
PRESS	10.90	Adeq Precision	12.160

The "Pred R-Squared" of 0.5706 is in reasonable agreement with the "Adj R-Squared" of 0.7328. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.160 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

Hardness = +3.90 +1.28 * A -0.81 * B -0.053* C +0.060* D

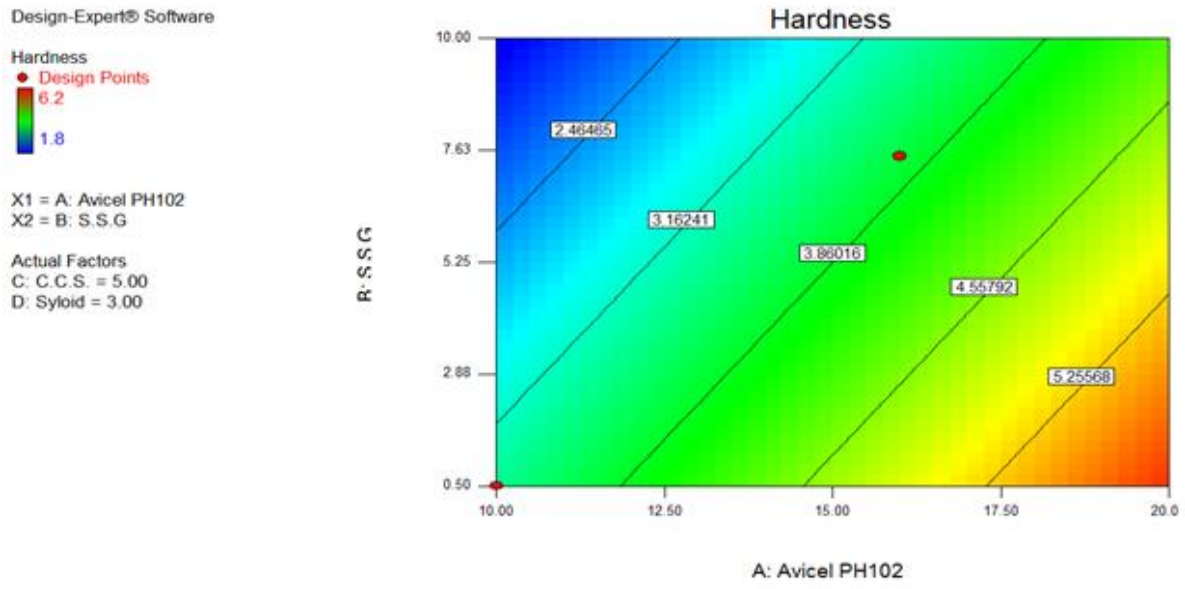


Figure Desirability plot (Contour plot) for Hardness

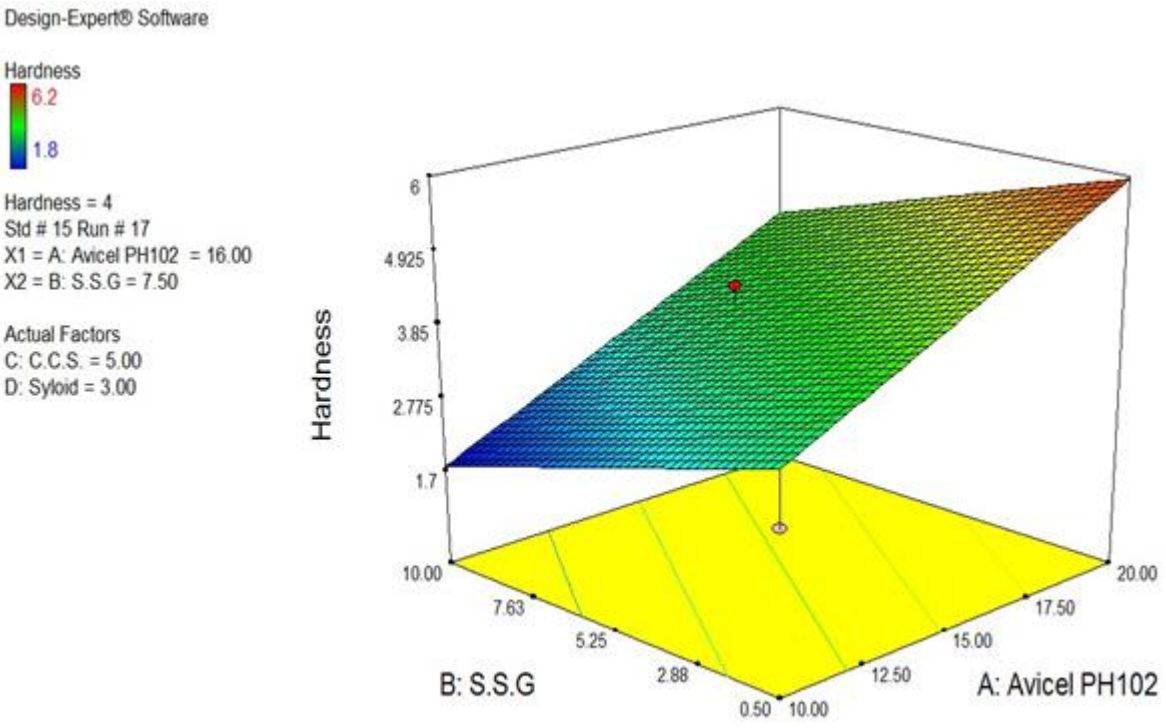


Figure Desirability plot (3D surface) for Hardness

3.4.3 Optimization of Formulation by Desirability Curve

For optimization of formulation, desirability curve was used. The upper and lower value was selected to prepare desirability curve.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Avicel PH	is target = 16.00	10	16	1	1	3
S.S.G	maximize	0.5	7.5	1	1	3
C.C.S.	maximize	0.5	5	1	1	3
Syloid	maximize	1	3	1	1	3
Disintegra	minimize	6.5	15	1	1	3
Hardness	maximize	1.8	6.2	1	1	3

Based on above limits, a solution was provided by the software which was considered as optimized formulation as shown below.

Optimized Tablet formulation having maximum desirability

Number	Avicel PH102	S.S.G	C.C.S.	Syloid	Disintegration	Hardness	Desirability
1	16.00	7.50	5.00	3.00	12.2111	3.73134	0.724
2	15.89	7.50	5.00	3.00	12.1418	3.7043	0.723
3	16.00	7.44	5.00	3.00	12.2625	3.74219	0.721
4	16.00	7.50	4.95	3.00	12.2434	3.73299	0.721
5	15.90	7.50	5.00	2.93	12.1721	3.70415	0.718
6	16.00	7.35	5.00	3.00	12.3405	3.75764	0.717
7	15.37	7.50	5.00	3.00	11.7996	3.57065	0.717
8	15.15	7.50	5.00	2.99	11.6576	3.51371	0.713
9	16.00	7.50	5.00	2.85	12.2724	3.72796	0.712
10	16.00	7.50	4.74	3.00	12.3683	3.73783	0.710
11	14.87	7.50	5.00	2.98	11.4772	3.44156	0.707
12	14.80	7.49	5.00	3.00	11.4356	3.42681	0.706
13	15.42	7.19	5.00	3.00	12.0899	3.636	0.706
14	16.00	7.50	4.59	3.00	12.4621	3.74144	0.702
15	14.83	7.50	5.00	2.88	11.4912	3.42821	0.698
16	16.00	7.50	5.00	2.68	12.3417	3.72355	0.697
17	15.77	7.26	5.00	2.75	12.3637	3.70789	0.692
18	14.17	7.50	5.00	3.00	11.0141	3.26283	0.690
19	14.90	7.50	5.00	2.69	11.6201	3.44273	0.684
20	13.73	7.50	5.00	3.00	10.7274	3.15069	0.677
21	14.98	6.53	5.00	3.00	12.36	3.63709	0.673
22	13.26	7.50	5.00	3.00	10.4184	3.02967	0.659
23	14.46	7.50	5.00	2.41	11.441	3.32088	0.650
24	16.00	7.50	3.98	2.45	13.0562	3.74211	0.620
25	15.61	6.47	5.00	2.31	13.1086	3.79225	0.612

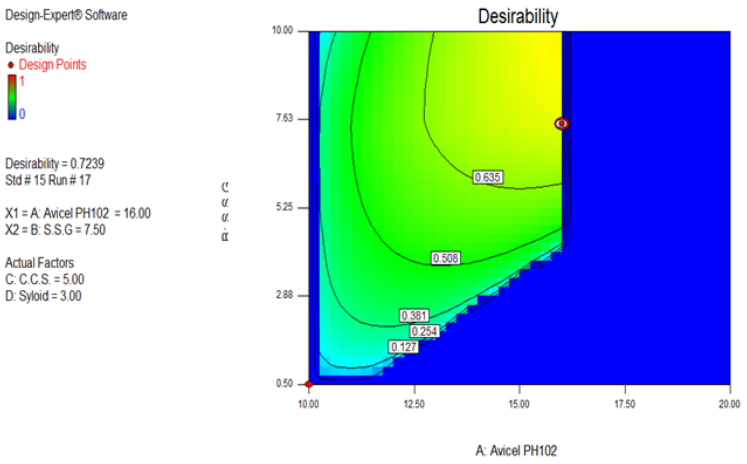


Figure Desirability plot (Contour plot) for optimized batch

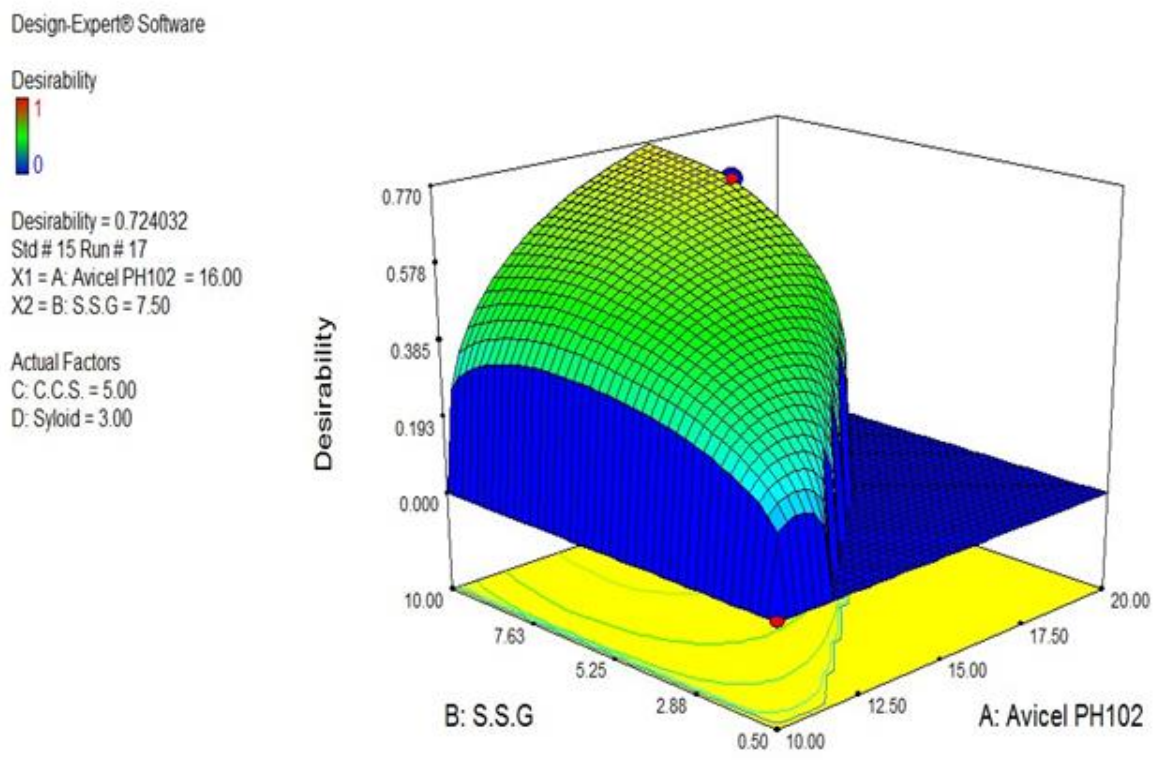
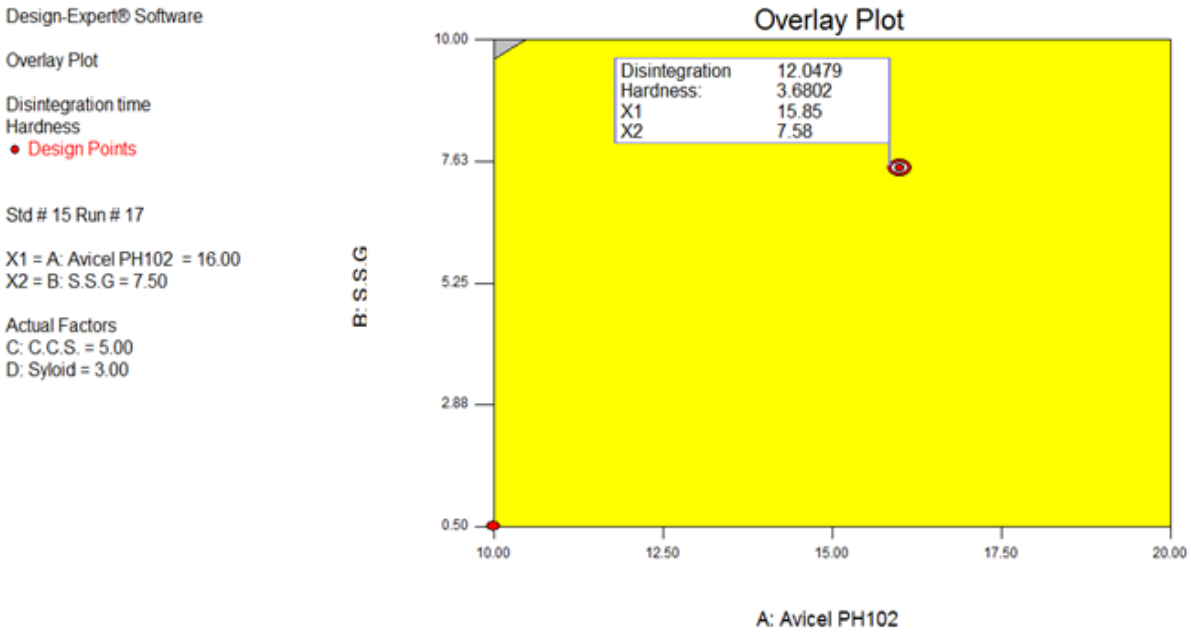


Figure Desirability plot (3D surface) for optimized batch

Overlay Plot

Overlay plot was plotted for creating a design space. Design space was created in such a way that the response would be between a particular limit.



Figure_Overlay plot for design space

Analysis of Design Space

It is very important to analyze the design space for robustness. This gives idea about reproducibility of results when formulation development is done in that particular design space. For analyzing the design space robustness, three formulations were flagged randomly in the plotted design space. The formulation was developed according to the provided values of factors and the observed responses were compared with predicted value of responses.

3.4.4 Check Point Batch Analysis:

Table_T-Test Table

Sr. No.	Disintegration Time		Hardness	
	Predicted value	Observed value	Predicted value	Observed value
1.	10.5	9.5	3.5	3.8
2.	10.5	10	3.5	3.5
3.	10.5	9.8	3.5	3.4
TTEST value	0.2176		0.6348	

From the robustness analysis, we observed that the observed responses were inside the boundary and the difference between predicted value and observed value was not significant. The above values of T-Test at 95 % of confidence interval complies within the standard value (4.3) Therefore model was validated.

Point Prediction & Confirmation:

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Avicel PH102	15.00	10.00	20.00	0.000	Actual
B	S.S.G	5.25	0.50	10.00	0.000	Actual
C	C.C.S.	2.75	0.50	5.00	0.000	Actual
D	Syloid	2.63	0.25	5.00	0.000	Actual

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Disintegration t	14.9683	0.26	14.40	15.54	0.93	12.95	16.99
Hardness	3.90396	0.19	3.49	4.32	0.68	2.43	5.38

Table showed predicted responses for the solution selected above along with the Standard deviation and 95 % confidence interval of the responses. Confirmation of the responses was done by carrying out the experiment using the selected factor values in triplicate. The table

showed and confirmed that experimental and predicted values were in good agreement concluding the suitability of the selected model for optimization.

3.4.5 Optimized Parameters for the formulation:

Table 4_ Optimized Excipients concentration

Ingredients	Concentration range	Taken Concentration
Avicel PH 102	10-20%	16%
Syloid	0.25-5%	3%
SSG	0.5-10%	7.5%
Cross carmellose sodium	0.5-5%	5%
Talc	1%	1%
Methyl paraben	1%	1%

Development of Tablet Formulation:

Tablet was prepared by Direct Compression method. Direct compression method is the best suitable method for developing polyherbal tablet.

In wet granulation method the mass became very sticky and in dry granulation the mass became too much hard difficult for disintegrating the tablet.

Syloid is having good adsorbant property. Therefore It is widely used in formulating polyherbal tablet. Here With the use of Syloid and without Syloid , comparison was made by appearing tablet.

Table_ Tablet Formulation

Achyranthus aspera ext.	50mg
Commiphora wightii ext	150mg
Morraya koinigi ext.	50mg
Garcinia indica ext	150mg
Avicel PH 102	100mg
Styloid	16mg
SSG	42mg
Cross carmellose sodium	30mg
Talc	6mg
Methyl peraben	6mg
Total	600mg

3.5 Evaluation Parameters for tablet:

Table 5_ Evalution of tablets

Appearance	Complies
Hardness	4.5 kg
Disintegration	9 mins
Friability	>1 %

3.6. Stability Study:

Stability study is used for the prediction of the shelf life of the herbal formulation. It is more important for the herbal formulation because it is very sensitive to moisture and microbial contamination. Tablet formulation was kept in sealed container and put in desiccators for 3 months. At the end of three month, the tablets were analysed according to physiological parameters.

Table_ Stability Data

Sr.No.	Physiological Parameters	Storage condition (Room Temperature)				
		0 Day	15 days	30 days	02 Months	03 Months
1	Appearance	Complies	Complies	Complies	Complies	Complies
2	Hardness(Kg)	4.5	4.5	4.3	4.3	4.3
3	Disintegration time (Minutes)	9	10	10	12	13
4	Friability (%)	0.8	0.8	0.85	0.85	0.85

3.7. Analytical method development and Validation:

Materials and Reagents:

Gallic acid was procured from Sulab (Suvidhinath) Laboratory, Vadodara and Oleanolic acid was procured from Sigma- Aldrich Company, USA. HPLC Grade Methanol and all other

reagents are obtained from Rankem Company. HPLC Grade Water is produced from Double distillation assembly at Laboratory throughout the whole study.

Instruments and equipment:

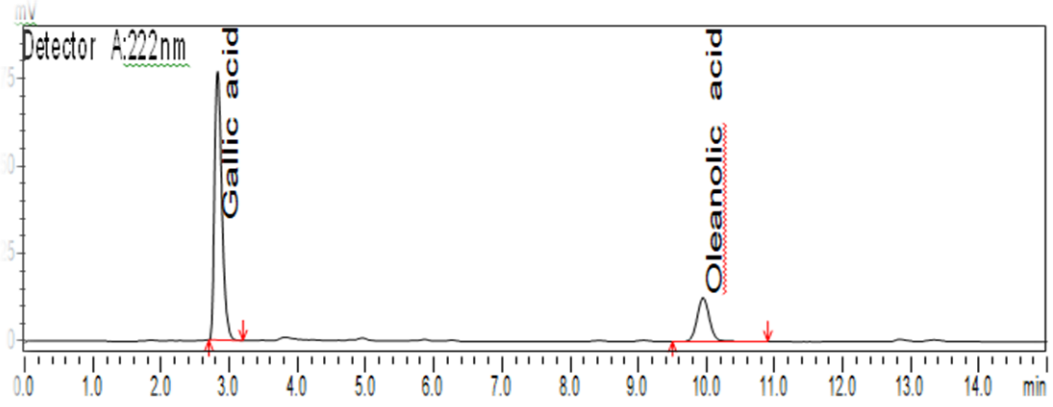
Weighing balance, UV Visible spectrophotometer, Sonicator, Hemilton Syringe 100 µl. Measuring cylinder, Mobile phase Reservior Bottle (Scott bottle)

HPLC instrumentation:

HPLC equipment	SHIMADZU LC-20AD Prominence
Column	Hyperchrom 5µ C18 (250 mm x 4.6 mm, 5 µm)
Detector	SHIMADZU SPD-20A Prominence UV/VIS Detector
Injector	Rheodyne 7725 injector valve with fixed loop at 20µl
Software	LC solution
System controller	SBM 20Alite

Method parameters:

Column	Hyperchrom ODS BP C18 (Size: 250*4.6 mm,5µ)
Flow rate	1.0 ml/min
Detection wavelength	222 nm
Mobile Phase	Ortho Phosphoric acid 0.1 % in Water : Methanol(5:95) It was filtered through 0.45 µm Nylon filter and sonicated for 5 min.
Injection Volume	20 µl through rheodyne manual injector.
Temperature	Ambient
Retention Time	2.8 min for Gallic acid and 9.9 min for Oleanolic acid



HPTLC Method Development and Validation:

Chemicals and Reagents:

Gallic acid was procured from Sulab (Suvidhinath) Laboratory, Vadodara and **Oleanolic acid** was procured from Sigma- Aldrich Company, USA. **E-Guggulsterone** was taken from Sigma Aldrich. HPLC Grade Methanol, Toluene, Ethyl acetate, Formic acid, Acetone was obtainedfrom Rankem company

HPTLC Instrumentation

Sample Applicator	Automated TLC sample applicator Linomat V with Nitrogen as inert gas
Syringe	Camag 100 µl HPTLC syringe
Development Chamber	Camag twin through chamber (10 x10 cm)
Scanner	Camag TLC Scanner
Software	Wincats software
HPTLC Plate	Aluminium plates pre coated with silica gel 60 F 254 (10 x 10 cm).The plates were activated at 110 0 C for 30 minutes Prior to chromatography.
Development chamber	Camag twin through glass chamber (20x20 cm). The optimized chamber saturation time for mobile phase was 45 Min at room temp.
Mobile Phase	Toluene : Ethyl Acetate : Formic acid (7:6:1)
Band width	6 mm
Length of chromatogram run	80 mm
Injection volume	20 µl
Detection wavelength	UV at 270 nm. 368 nm after spraying with Methanolic Sulphuric acid.

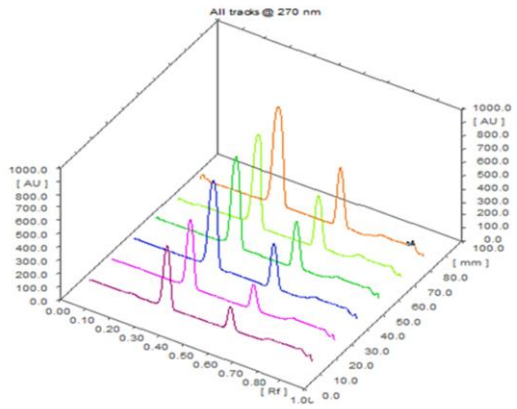
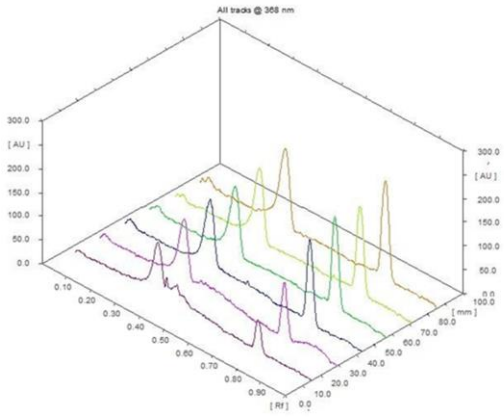


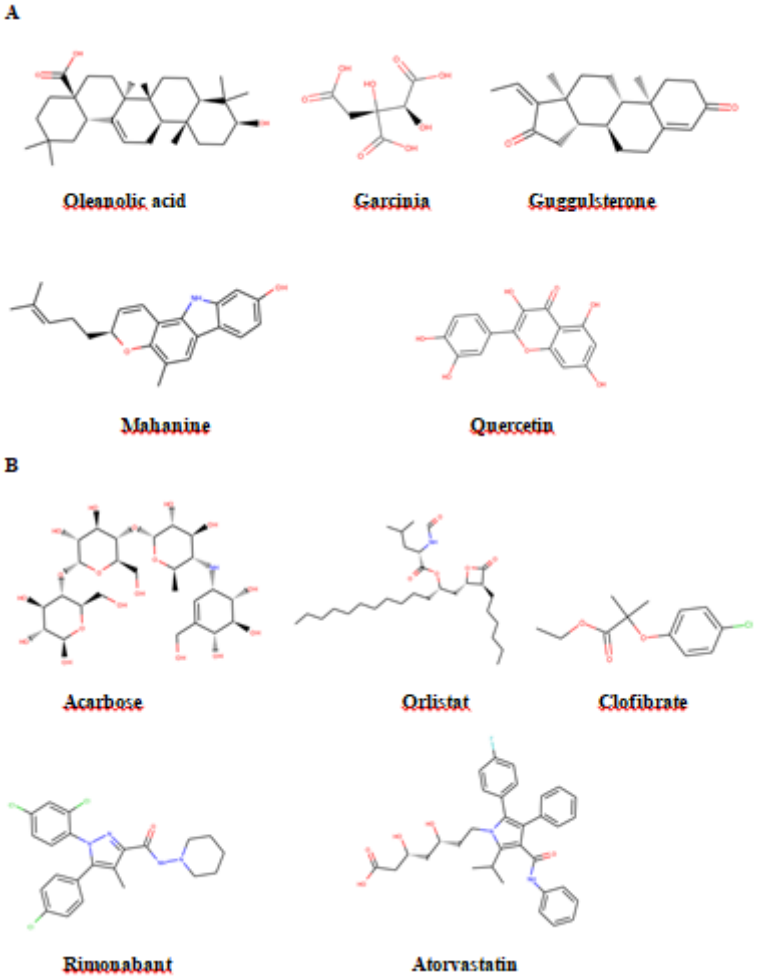
Figure_ HPTLC graph for linearity of Gallic acid and E-Guggulosterone scannd at 270 nm



Figure_HPTLC chromatogram for Linearity of Oleanolic acid scanned at 368 nm.

3.8. Anti-obesity evaluation of markers present in selected herbs using *In Silico* Approach.

Markers and selected class of drugs for evaluation



8.1 Pancreatic α-Amylase (Inhibitory activity):

Molecular Docking Study:

Identification of Active site of α-Amylase:

The PDB structure (6GXV) of the AliC GH13 alpha-amylase from *Alicyclobacillus* sp. from www.rcsb.org has been selected for the molecular docking activity. Co Crystallized structure of alpha amylase is available in complex with alpha-glucosidase inhibitors Acarbose. A good resolution (2.07 A) X-Ray diffraction protein structure showed interactions with D-glucopyranose. Based on the interactions of glucopyranose observed with the protein structures, active site of the protein structure has been identified. The identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina:

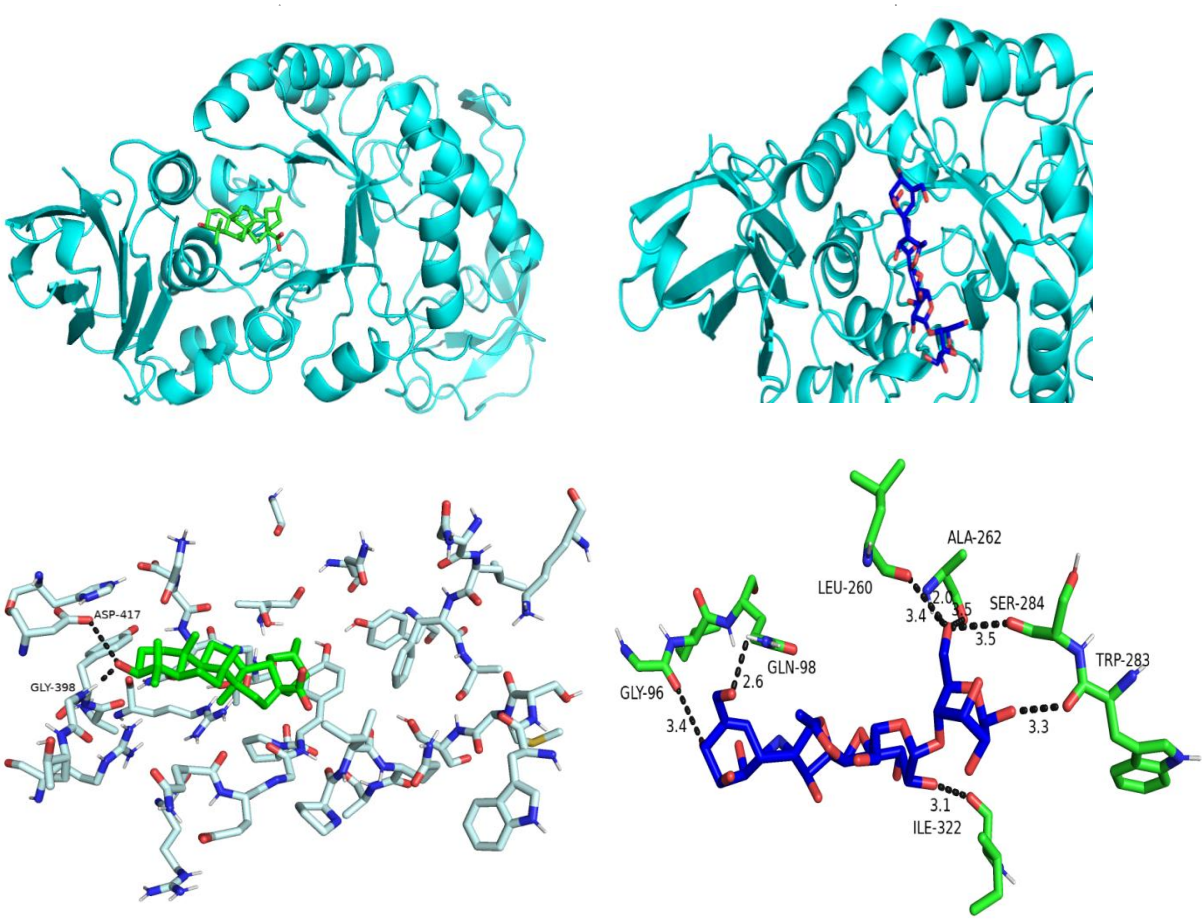
To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs), molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of alpha-amylase was obtained from the protein data bank (PDB id: 6GXV, resolution: 2.07 Å). Autodock MGL Tool was used to modify the protein (alpha-amylase) and ligands (NPs and Drug molecules) structures. The protein (alpha-amylase) was prepared by removing water, unwanted chain and a bound ligand. Missing amino acids have been checked, and the protein structure has been added with polar hydrogen and Kollman charges. After which Grid box was prepared according to the identified active site. Center Grid box was selected using x: 66.817, y: 24.937, z: -6.517, and the number of points in all dimensions x,y,z were considered 40x40x40 Å , and the grid spacing was selected as 0. 50 Å.

Molecular Docking Study:

The PDB file (6GXV) of the Crystal Structure of the AliC GH13 alpha-amylase was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 1 gives the binding energies of all active constituents of natural products and standard drugs with 6GXV (binding energies ranged from –9.0 to -4.7 kcal/mol).

Table_ Molecular docking results of NPs and some standard drugs with alpha-amylase (6GXV)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	**Oleanolic acid**	**-9.0**
2	Garcinia	-4.7
3	Guggulsterone	-7.6
4	Mahanine	-8.1
5	Quercetin	-7.6
6	**Acarbose**	**-7.6**
7	Orlistat	-5.3
8	Clofibrate	-5.1
9	Rimonabant	-7.9
10	Atorvastatin	-8.5



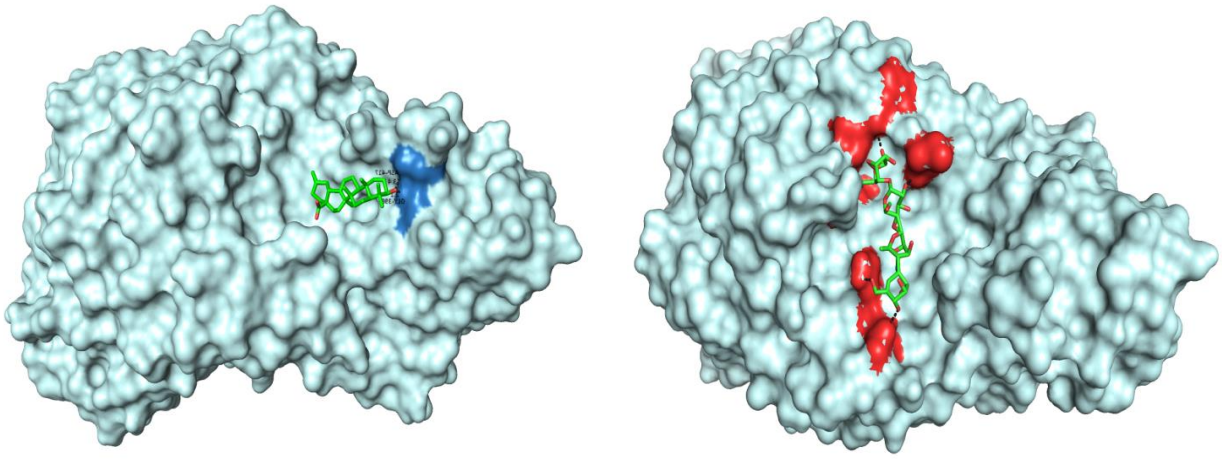


Figure_ Docking interactions of (A) Oleanolic acid and (B) Acarbose in the active sites of alpha-amylase (PDB id: 6GXV)

Oleanolic acid bound efficiently to the active site of alpha-amylase with good complementarity, and the docking score is -9.0 kcal/mol. which is far more compared with standard drug acarbose (docking score -7.6 kcal/mol). The Oleanolic acid binds to alpha-amylase by two hydrogen bond with GLY-398 and ASP-417 other nonbonding hydrophobic interactions. Moreover, mahanine has also showed comparative binding with alpha-amylase (Figure 5.23). Among the all standard drugs used for docking, atorvastatin showed comparative binding to alpha-amylase protein. However, identifying the ligand binding site (composed of amino acids) for each specific protein molecule is crucially important when trying to find a suitable drug molecule for the target. It is also essential to understand the function of the protein. These binding interactions (Figure 5.23) present a clear view that Oleanolic acid can have good interactions with alpha-amylase.

3.8.2Pancreatic Lipase:

Identification of Active site of human gastric lipase

The PDB structure (1HLG) of recombinant human gastric lipase with the resolution of 3.0-Å was selected which is described under mammalian acid lipase family. The Crystallized structure of human gastric lipase is available in complex with 2-acetamido-2-deoxy-beta-D-glucopyranose. Based on the interactions of glucopyranose observed with the protein structures, active site of the protein structure has been identified. The identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina

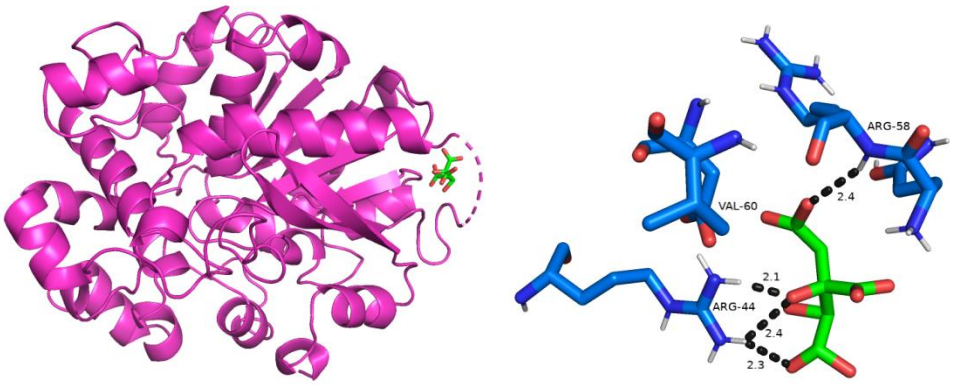
To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs), molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of human gastric lipase was obtained from the protein data bank (PDB id: 1HLG). Autodock MGL Tool was used to modify the protein (human gastric lipase) and ligands (NPs and Drug molecules) structures. The protein (human gastric lipase) was prepared by removing water, unwanted chain and a bound ligand. Missing amino acids have been checked, and the protein structure has been added with polar hydrogen and Kollman charges. After which Grid box was prepared according to the identified active site. Center Grid box was selected using x: 51.910, y: 70.449, z: 82.856, and the number of points in all dimensions x,y,z were considered 40x40x40 Å , and the grid spacing was selected as 0. 50 Å.

Molecular Docking Study

The PDB file (1HLG) of recombinant human gastric lipase was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 2 gives the binding energies of all active constituents of natural products and standard drugs with 1HLG (binding energies ranged from –8.4 to -4.7 kcal/mol).

Table_ Molecular docking results of NPs and some standard drugs with human gastric lipase

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.4
2	**Garcinia**	**-4.7**
3	Guggulsterone	-7.6
4	Mahanine	-6.4
5	Quercetin_meletin	-7.2
6	Acarbose	-7.2
7	**Orlistat**	**-4.8**
8	Clofibrate	-5.1
9	Rimonabant	-7.1
10	Atorvastatin	-7.1



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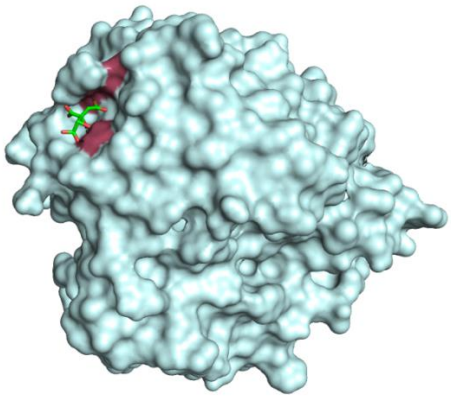

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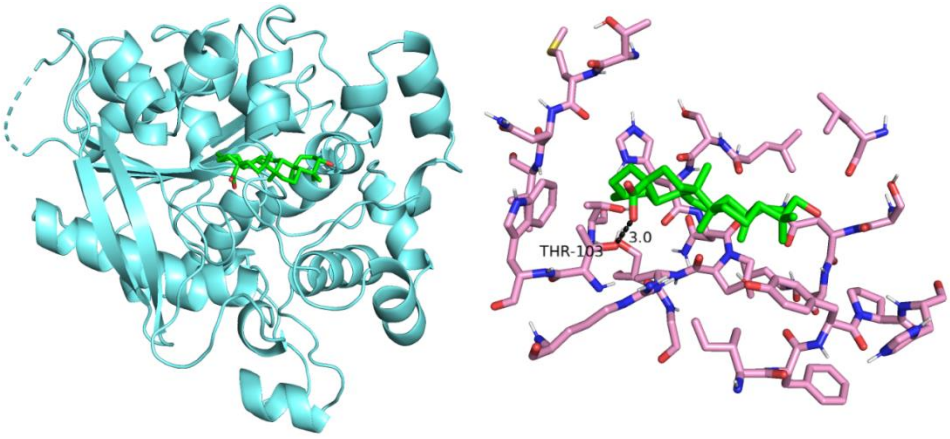

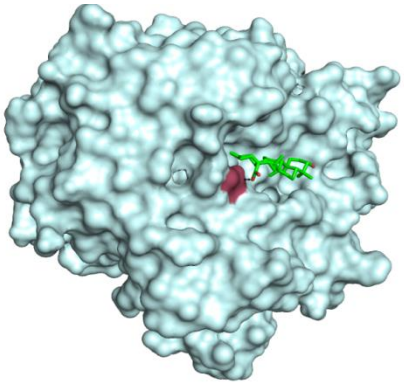

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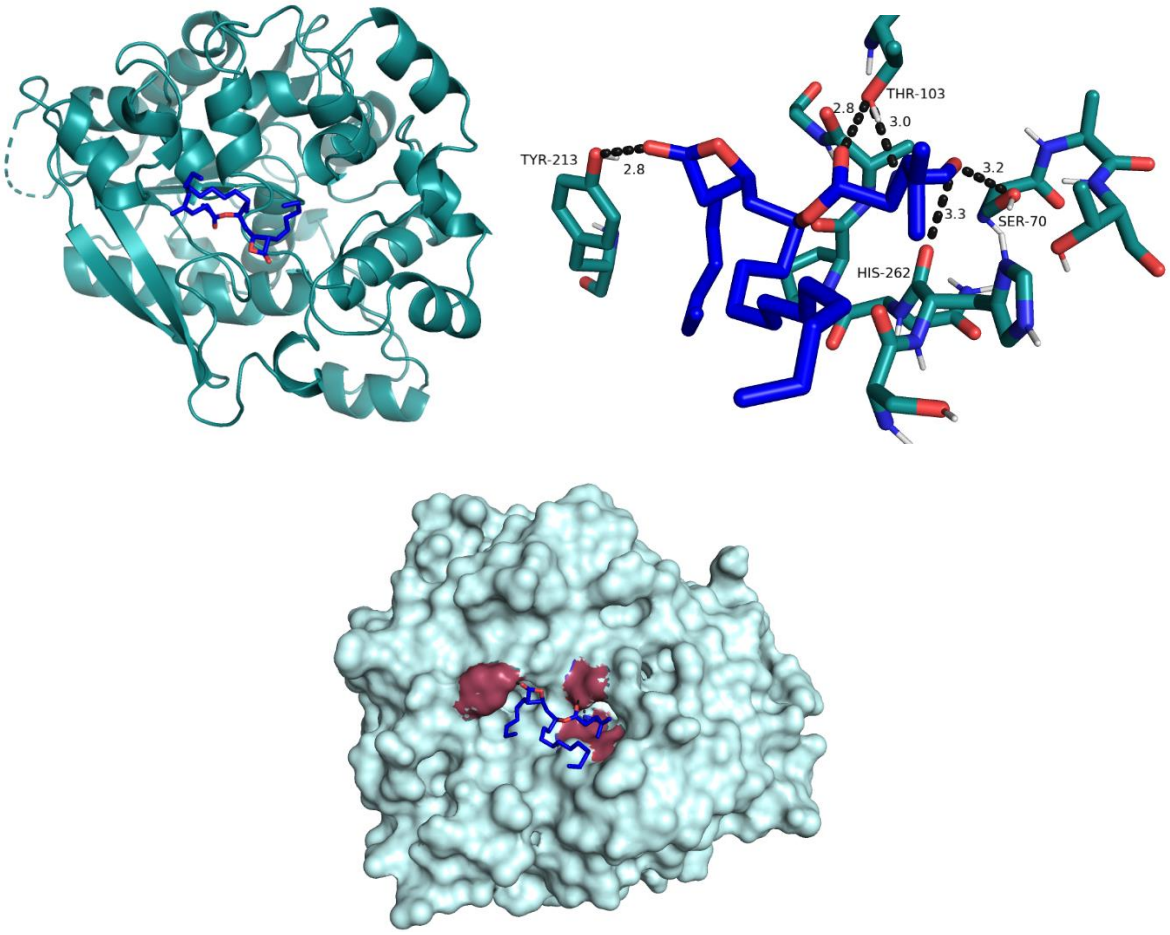

Figure_ Docking interactions of (A) Garcinia (B) Oleanolic acid and (C) Orlistat in the active sites of human gastric lipase (PDB id: 1HLG)

Garcinia showed some interactions with human gastric lipase protein through hydrogen bonding and other nonbonding interactions but the interactions are not strong enough as the docking score is -4.7 kcal/mol while Oleanolic acid shoed good binding interection with docking score of -8.4 kcal/mol (Table 2).

3.8.3 PPARs (peroxisome proliferator activated receptor) (PPARalpha):

Identification of Active site of Human Peroxisome proliferator-activated receptors (PPARs) alpha

The PDB structure (3VI8) of Human Peroxisome proliferator-activated receptors (PPARs) alpha from www.rcsb.org has been selected for the molecular docking activity. The protein is Co Crystallized structure with a synthetic agonist APHM13. A good resolution (1.75 Å) X-Ray diffraction protein structure showed interactions with agonist APHM13. Based on the interactions of agonist APHM13 with the protein structures, active site of the protein structure has been identified. The identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina

To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs), molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of Human PPAR alpha was obtained from the protein data bank (PDB id: 3VI8). Autodock MGL Tool was used to modify the protein (alpha-amylase) and ligands (NPs and Drug molecules) structures.

Molecular Docking Study:

The PDB file (3VI8) of human PPAR alpha was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 3 gives the binding energies of all active constituents of natural products and standard drugs with 3VI8 (binding energies ranged from –9.9 to -4.3 kcal/mol).

Table_ Molecular docking results of NPs and some standard drugs with human PPAR alpha (3VI8)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.0
2	Garcinia	-5.9
3	**Guggulsterone**	**-7.9**
4	Mahanine	-8.5
5	Quercetin	-9.5
6	Acarbose	-5.7
7	Orlistat	-4.3

8	**Clofibrate**	**-6.2**
9	Rimonabant	-9.9
10	Atorvastatin	-6.3

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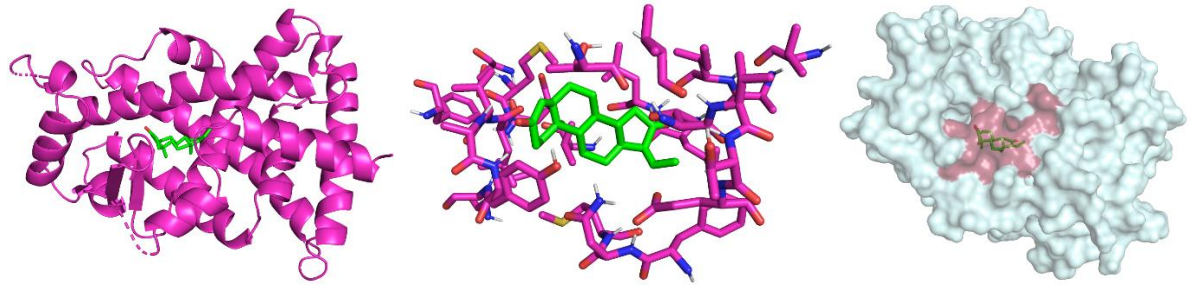

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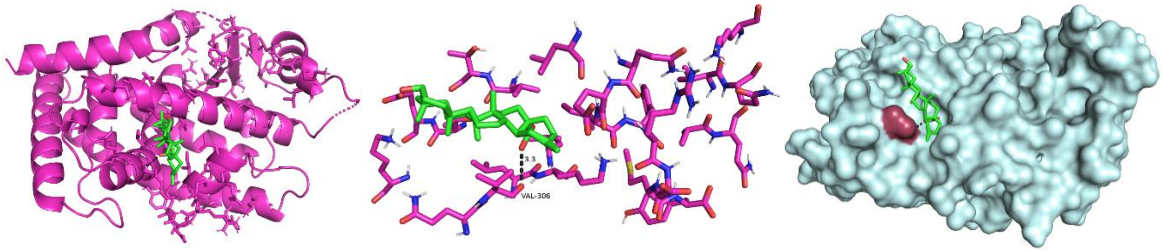

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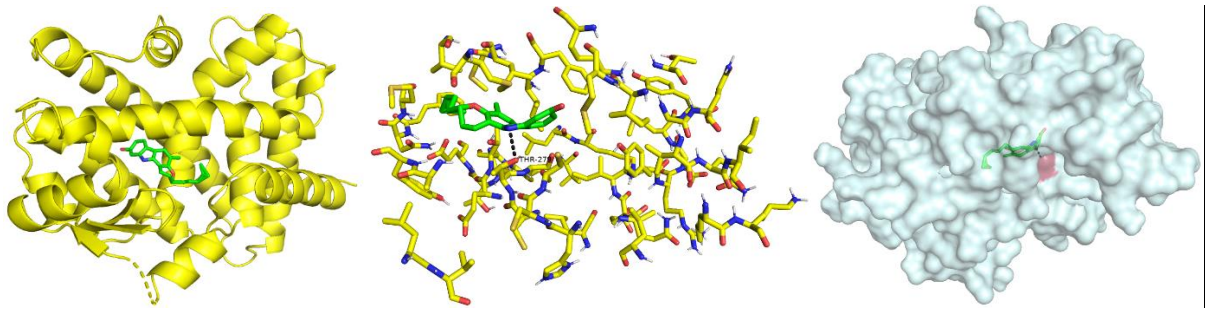

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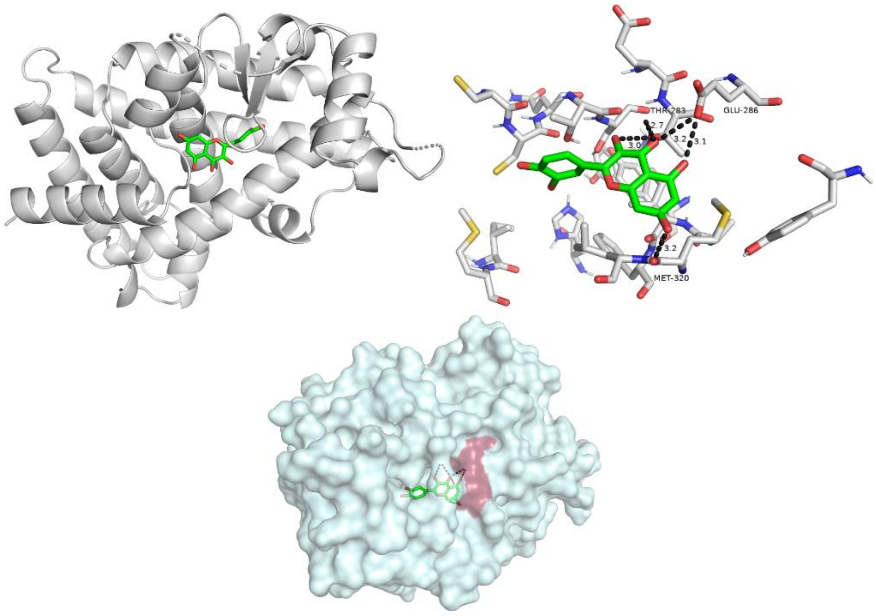

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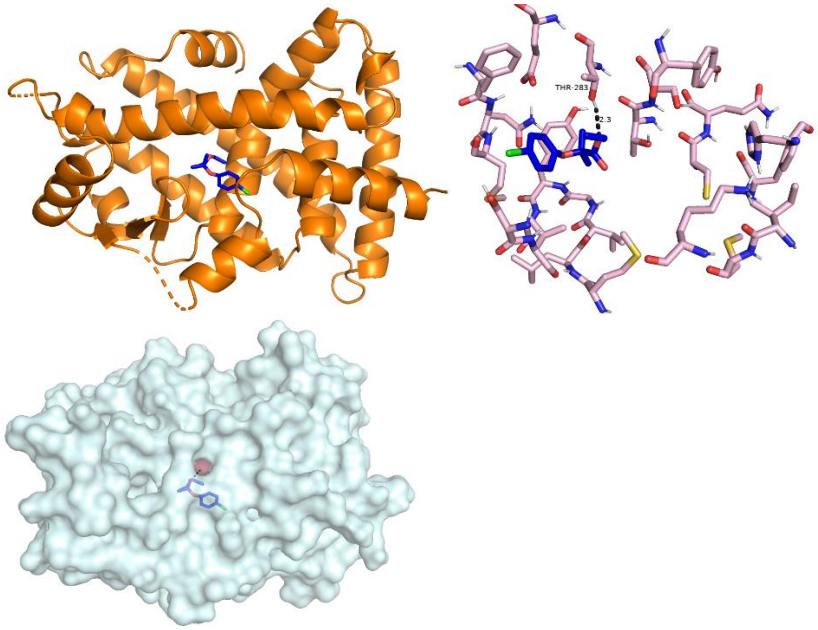

Figure_Docking interactions of (A) Guggulsterone (B) Oleanolic acid (C) Mahanine (D) Quercetin and (E) Clofibrate in the active sites of human PPAR alpha (PDB id: 3VI8)

Guggulsterone, Oleanolic acid, Mahanine, and Quercetin showed good binding interactions with the human PPAR alpha efficiently as the docking score is -7.9, -8.0, -8.5, and -9.5 kcal/mol, respectively (Table 3). Moreover, all the natural compounds have formed many hydrogen bonding with the active site of human PPAR alpha amino acids (Figure 4) along with hydrophobic interactions. To find a suitable drug molecule for the target, the binding site was identified in proper way to eliminate false results. The binding interactions (Figure 4)

present a clear view that the NPs like Guggulsterone, Oleanolic acid, Mahanine, and Quercetin can irreversibly interact with human PPAR alpha. Bond distance and binding interaction of Guggulsterone, Oleanolic acid, Mahanine, and Quercetin with an active amino acid of human PPAR alpha are less than 3 Å indicatind stronger interactions. On the other side the selected drugs don't have good binding interactions (docking score ranges from -4.3 to -6.3 kcal/mol) except Rimonabant (docking score -9.9 kcal/mol). The reported drug Clofibrate has showed docking score of only -6.2 kcal/mol indicating poor interactions compared to NPs.

3.8.4 Leptin (LEP-R, LEP-Rb):

Identification of Active site of human obesity protein, leptin

The PDB structure (1AX8 and 3V6O) of human obesity protein, leptin and leptin receptor-antibody complex from www.rcsb.org has been selected for the molecular docking activity. The aim was to consider Leptin receptor and its antibody complex using cocrystallized structure. The selected good resolution (2.40 Å and 1.95 Å) X-Ray diffraction protein structure showed interactions with monoclonal antibody 9F8 fab fragment Heavy chain and 2-acetamido-2-deoxy-beta-D-glucopyranose. Based on the interactions, active site of the protein structure has been identified and the identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina

To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs), molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of human obesity protein, leptin and leptin receptor-antibody complex were obtained from the protein data bank (PDB id: 1AX8 and 3V6O). Autodock MGL Tool was used to modify the protein (human obesity protein, leptin and leptin receptor-antibody complex) and ligands (NPs and Drug molecules) structures

Molecular Docking Study

The PDB file (1AX8 and 3V6O) of human obesity protein, leptin and leptin receptor-antibody complex were downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding

interactions of NPs and drugs with protein. Moreover, Table 4 gives the binding energies of all active constituents of natural products and standard drugs with 1AX8 (binding energies ranged from –3.9 to -7.9 kcal/mol) and with 3V6O (binding energies ranged from –4.3 to -7.5 kcal/mol).

Table_ Molecular docking results of NPs and some standard drugs with leptin (1AX8)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-7.5
2	Garcinia	-4.3
3	Guggulsterone	-6.4
4	**Mahanine**	**-6.4**
5	Quercetin	-6.4
6	Acarbose	-6.3
7	Orlistat	-4.4
8	Clofibrate	-4.3
9	Rimonabant	-6.6
10	Atorvastatin	-6.6

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Table_Molecular docking results of NPs and some standard drugs with leptin (3V6O)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-7.9
2	Garcinia	-4.2
3	Guggulsterone	-6.9
4	**Mahanine**	**-6.4**
5	Quercetin	-7.0
6	Acarbose	-6.8
7	Orlistat	-3.9
8	Clofibrate	-4.4
9	Rimonabant	-7.1
10	Atorvastatin	-6.9

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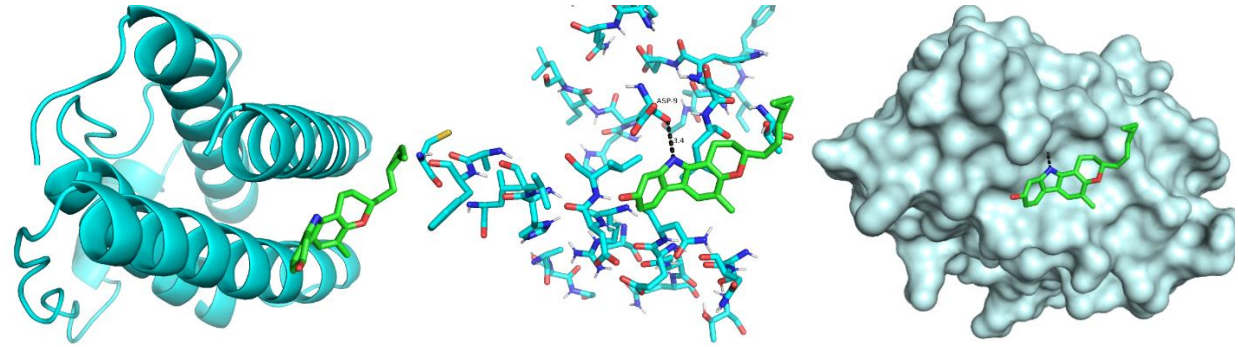

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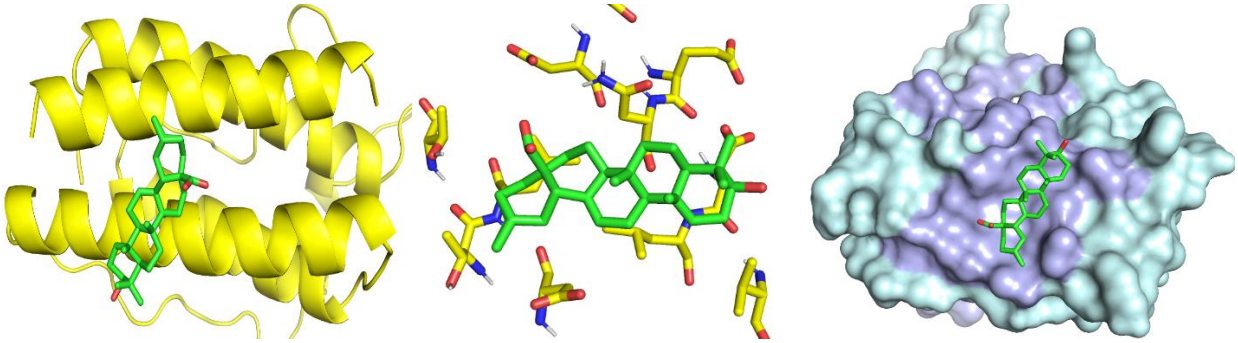

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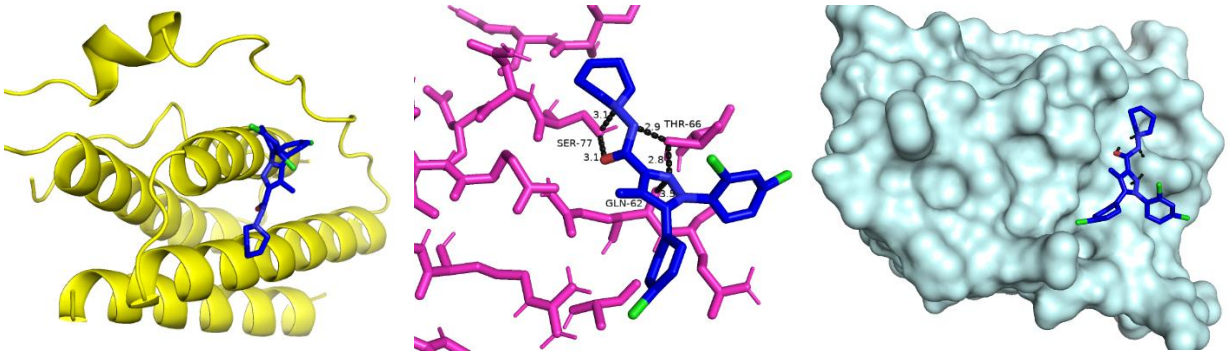
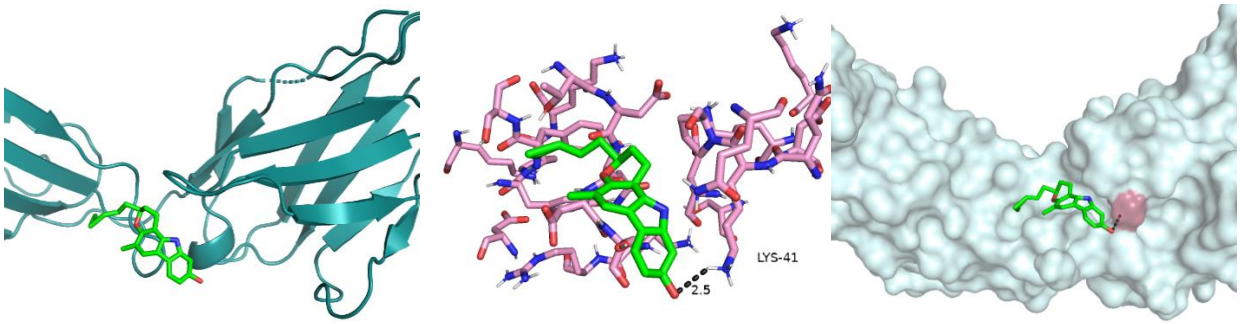


Figure_ Docking interactions of (A) Mahanine (B) Oleanolic acid (C) Rimonabant in the active sites of human obesity protein, leptin (PDB id: 1AX8)

A



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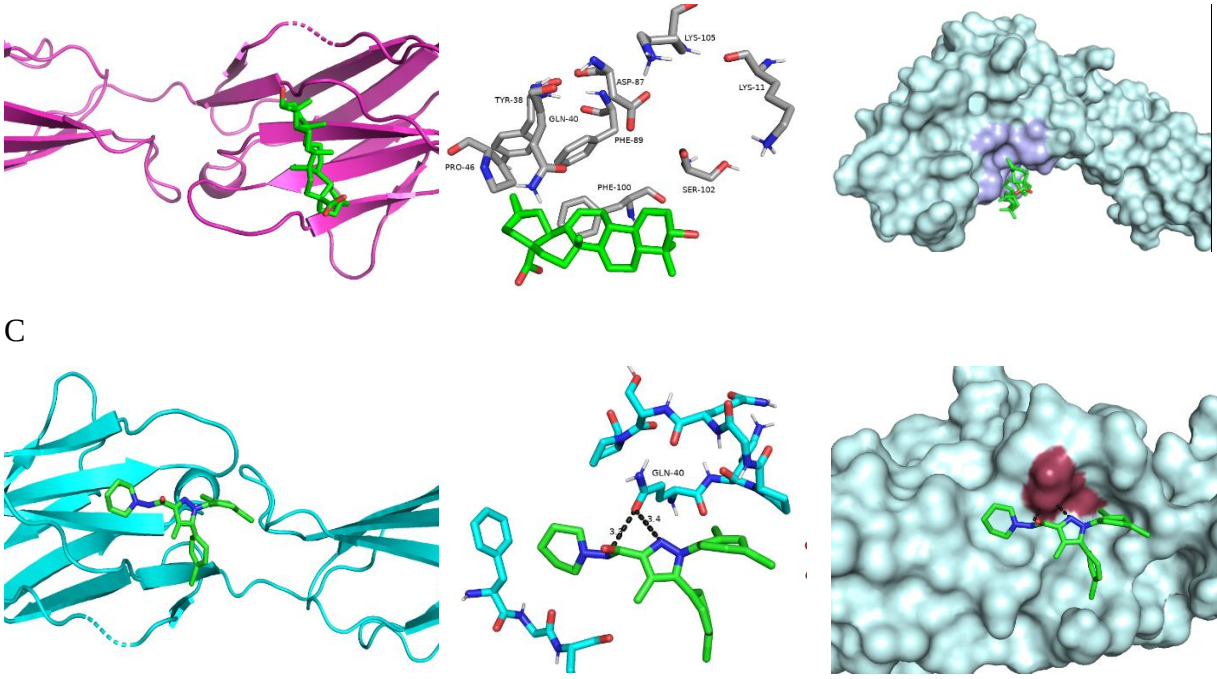

FigurE_Docking interactions of (A) Mahanine (B) Oleanolic acid (C) Rimonabant in the active sites of leptin receptor (PDB id: 3V6O)

Amongst all the NPs, Mahanine and Oleanolic acid are showing interactions with the active site of human obesity protein, leptin with good complementarity, and the docking score is -6.4 and -7.9 kcal/mol, respectively for 1AX8 whereas -6.4 and -7.5 kcal/mol, respectively for 3V6O (Table 4 and Table 5). Moreover, the drug Rimonabant also showing bonding interactions with the active site of leptin with the docking score of -7.1 kcal/mol for 1AX8 (Figure 5) whereas -6.6 kcal/mol, for 3V6O (Figure 6). The pose and interaction suggested that the oleanolic acid binds strongly with the leptin in all the protein structures.

3.8.5 Cannabinoid receptor type 1(CB1):

Identification of Active site of Cannabinoid receptor type 1(CB1)

The PDB structure (7V3Z) of Cannabinoid receptor type 1(CB1) from www.rcsb.org has been selected for the molecular docking activity. The structure of cannabinoid receptor type 1 is available in complex with small molecules like flavin mononucleotide, cholesterol and 2-[(1r,2r,5r)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol. Based on the interactions of small molecules with the protein structure, active site of the protein structure has been identified. The identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina

To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs) and selected drug molecules, molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of cannabinoid receptor type 1 was obtained from the protein data bank (PDB id: 7V3Z). Autodock MGL Tool was used to modify the protein (cannabinoid receptor type 1) and ligands (NPs and Drug molecules) structures.

Molecular Docking Study

The PDB file (7V3Z) of the cannabinoid receptor type 1 was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 6 gives the binding energies of all active constituents of natural products and standard drugs with 7V3Z (binding energies ranged from –4.4 to -8.2 kcal/mol).

Table_ Molecular docking results of NPs and some standard drugs with cannabinoid receptor type 1 (7V3Z)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.2
2	Garcinia	-4.8
3	Guggulsterone	-7.8
4	Mahanine	-7.9
5	**Quercetin**	**-8.1**
6	Acarbose	-6.3
7	Orlistat	-4.4
8	Clofibrate	-6.2
9	**Rimonabant**	**-7.1**
10	Atorvastatin	-7.0

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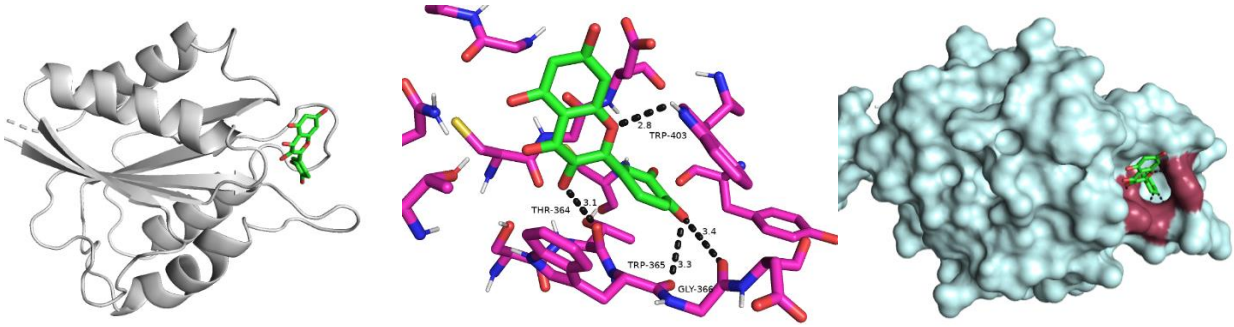

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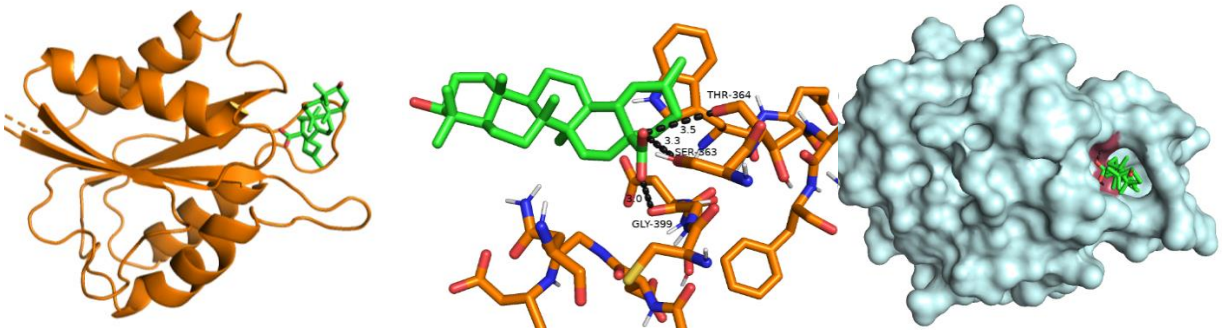

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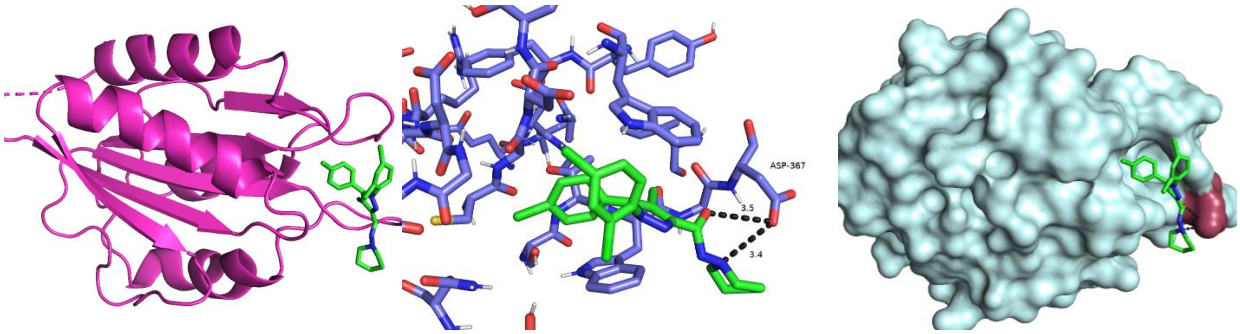


Figure_ Docking interactions of (A) Quercetin (B) Oleanolic acid (C) Rimonabant in the active sites of cannabinoid receptor type 1 (PDB id: 7V3Z)

Quercetin, and oleanolic acid are bound efficiently to the active site of cannabinoid receptor type 1 with good complementarity, and the docking score is -8.1 and -8.2 kcal/mol, respectively (Table 2). All the selected drug molecules showed comparatively low docking score than quercetin, and oleanolic acid. Moreover, the reported drug rimonabant also shows less binding interactions with docking score of -7.1 kcal/mol. Quercetin, and oleanolic acid showed four and three hydrogen bond with the amino acid THR 364, TRP 365, GLY 366, TRP 403 and THR 364, SER 363, GLY 399 respectively from cannabinoid receptor. In

comparison to this the drug rimonabant only have two hydrogen bond with the amino acid ASP 367 of cannabinoid receptor. These binding interactions (Figure 7) present a clear view that quercetin, and oleanolic acid can irreversibly interact cannabinoid receptor type 1. Binding interaction of quercetin, and oleanolic acid can irreversibly interact cannabinoid receptor type 1 are outlined in above given table.

3.8.6 HMG CoA Reductase:

Identification of Active site of human HMG-CoA reductase

The PDB structure (1DQA) of the human HMG-CoA reductase from www.rcsb.org has been selected for the molecular docking activity. Co Crystallized structure of human HMG-CoA reductase is available in complex with HMG, CoA, and NADP+. A good resolution (2.00 Å) X-Ray diffraction protein structure showed interactions with HMG, CoA, and NADP+. Based on the interactions observed with the protein structures, active site of the protein structure has been identified. The identified active site has been again confirmed with the online server 3D Ligand Site (https://www.wass-michaelislab.org/3dlig/index.html).

Molecular Docking using Autodock Vina

To obtain details about the binding interaction as well as the relative orientation of the natural products (NPs), molecular docking was used. To obtain the comparative results, the standard drugs are also considered for the molecular docking. The protein structure of human HMG-CoA reductase was obtained from the protein data bank (PDB id: 1DQA). Autodock MGL Tool was used to modify the protein (human HMG-CoA reductase) and ligands (NPs and Drug molecules) structures.

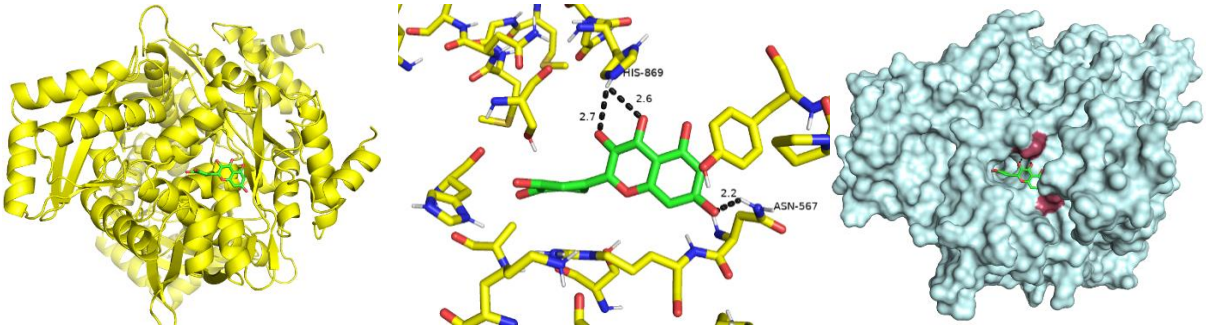
Molecular Docking Study

The PDB file (1DQA) of the catalytic portion of human HMG-CoA reductase with HMG, CoA, and NADP+ was downloaded from the PDB databank and ligands were downloaded from the ZINC database (Figure.1). Autodock Vina software was used to predict the binding interactions of NPs and drugs with protein. Moreover, Table 7 gives the binding energies of all active constituents of natural products and standard drugs with 1DQA (binding energies ranged from –8.9 to -5.2 kcal/mol).

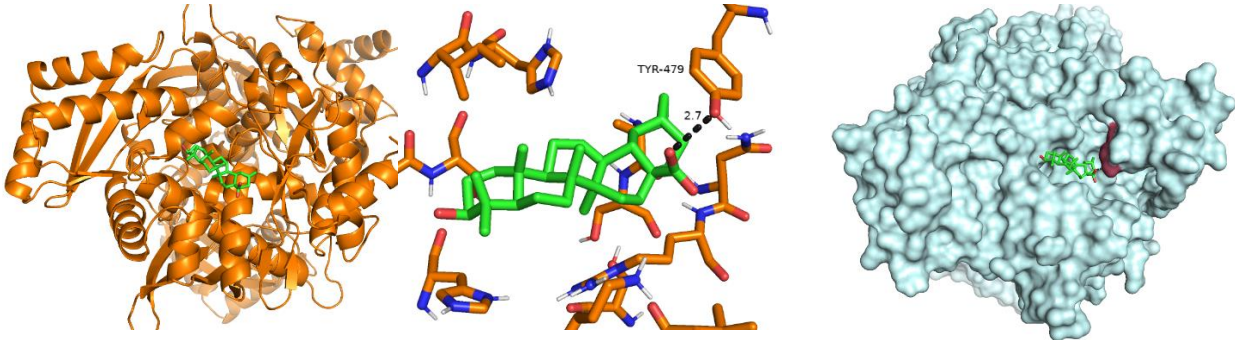
Table _ Molecular docking results of NPs and some standard drugs with catalytic portion of human HMG-CoA reductase (1DQA)

Sr. No	Marker Compound and Drugs	Affinity (kcal/mol)
1	Oleanolic acid	-8.9
2	Garcinia	-5.9
3	Guggulsterone	-8.1
4	Mahanine	-8.3
5	**Quercetin**	**-8.2**
6	Acarbose	-8.3
7	Orlistat	-5.4
8	Clofibrate	-5.2
9	Rimonabant	-8.9
10	**Atorvastatin**	**-8.2**

A



B



C

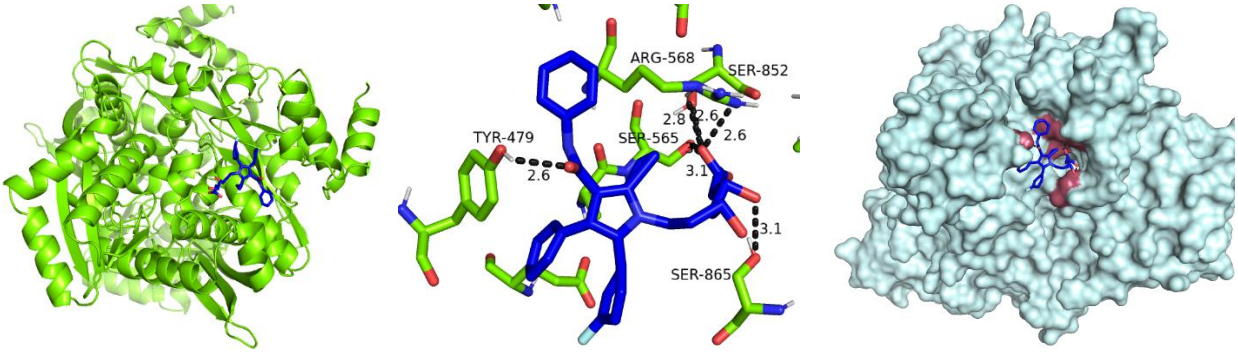


Figure_ Docking interactions of (A) Quercetin (B) Oleanolic acid (C) Atorvastatin in the active sites of human HMG-CoA reductase (PDB id: 1DQA)

Quercetin, oleanolic acid, guggulsterone, and mahanine, are bound efficiently to the active site of human HMG-CoA reductase with good complementarity, and the docking score is -8.2, -8.9, -8.1 and -8.3 kcal/mol, respectively. Moreover, oleanolic acid has highest interactions with the active site of human HMG-CoA reductase, while other NPs also showed significant docking score for the binding with protein. In the selected drugs, Rimonabant, and Atorvastatin have shown good binding interactions with the docking score of -8.9 and -8.2 kcal/mol, respectively (Table 7). Quercetin is forming three hydrogen bond with ASN 567 and HIS 869 while oleanolic acid formed one H-bond with TYR 479. Also quercetin, and oleanolic acid formed many nonbonding interaction with protein. The standard drug atorvastatin formed many H-bonds but has less nonbonding interactions. All the interactions can be visualized from the figure.

4. Summary and Conclusion.

Summary & Conclusion:

As per World Health Organization: Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness". The core use of herbal medicines is for health promotion and treatment for chronic, as different to life-threatening, conditions. However, usage of traditional remedies rises when conventional medicine is ineffective in the treatment of disease like obesity.

Consistent and safe herbal product for weight reduction is a need of developed and developing countries. In our literature survey, herbal plants showed potential effects on weight control. However, for the majority of products, more data are needed to assess the suitability as anti-obesity plants**.**

Everyone knows that exercise with a controlled diet is the only way to keep in shape. However, your aim to be slim is obstructed by your urge to eat more and to snack in between meals. It's difficult for many people to resist food or snacks after a long and tiring work day. It's only natural! Tiredness and fatigue can also make people crave sugary food for energy. That's why many are unable to stick to healthy food choices every day. But now imagine the same situation with a controlled appetite. With your appetite under your control, you can lower the intake of calories.

To overcome the side effects of the current allopathic treatment, Herbal will be seen as the alternative medication.

Obese persons are preferred the use of herbal products for weight management because of following probable reasons:

- ✓ Health benefits of weight loss without any side effects,
- ✓ Less demanding than accepted lifestyle changes, such as exercise and diet,
- ✓ Easily available without a prescription,
- ✓ More easily accepted than a professional consultation with a physician or a nutritionist
- ✓ 100% natural origin and perception that natural means safe
- ✓ Herbal plants for weight reduction may be effective in the treatment of obesity and associated disorders.

Herbal medicines are playing a significant role in preservation of health in rural and remote areas, and provide the health for all. Utilization of Herbal medicine along with conventional drug defiantly helps health or cure diseases in the positive way. Herbal Drug contains the lots of Chemical moieties, which will help to inhibit the whole pathway of lipid synthesis.

Herbal plant selected based on their individual targets for reduction of obesity. The pathogenesis of obesity involves regulation of calorie utilization, appetite, and physical activity, but has complex interactions with availability of health-care systems, the role of socio-economic status, and underlying hereditary and environmental factors.

*Achyranthus aspera(*Aghedo seeds)*, Garcinia Indica fruit (Kokam fruits), Murraya koeinigii (*Curry patta)and *Commiphora mukul* (Guggul) extracts having the known Anti-obesity activity.

Individual selected plants are having their own mechanism to treat the obesity.

Achyranthes aspera seed can inhibit obesity by reducing the excess accumulation of body fat and altering the serum lipid profile. This is because of marker compound like oleanolic acid. In Ayurveda mention that consumption of seeds of achyranthes aspera is suppress the appetite. And that's how used to treat the obesity.

Executive Summary

Extracts of *Murraya koenigii* leaves significantly reduced the body weight gain, plasma total cholesterol (TC) and triglyceride (TG) levels significantly due to presence of various alkaloids.

Garcinia Indica Besides its efficacy in the decrease of body weight and food intake, Garcinia has been proven to be beneficial in ameliorating obesity-related problems such as inflammation, oxidative stress, and insulin resistance. Hydro citric acid and other reported phenolic compounds predicted as reduce the obesity.

Commiphora mukul has been used in Ayurvedic medicine for many years to treat a variety of ailments like obesity, lipid disorders, rheumatoid arthritis, bone fracture, cardiovascular disorder disease and antihyperglycemic and antioxidant activities.

Phytochemical screening was done to check the presence of various primary and secondary metabolites likes proteins, carbohydrates, flavonoids, alkaloids and glycosides.

Quantitative analysis was also done to verify the presence of various amounts of flavonoids and phenolics compounds. As we know that this flavonoids and phenolics compounds can play an important role as antioxidant. These present antioxidants having significant role in the treatment of obesity.

Here Tablets were prepared by Direct Compression method. Direct compression method is the best suitable method for developing herbal tablet.

The whole study was focused on the development of the formulation via using DOE with the appropriate concentration of the different excipients which, passes all preliminary and secondary parameters of the formulations like Hardness, Friability and Disintegration time.

Avicel PH 102, Syloid, Cross carmellose sodium, talc, Methyl and propyl paraben were the selected excipients from the trial and error method. Syloid was the majorly focused excipient just because of the moisture sensitivity of the drug.

Physical Stability at room temperature was evaluated as per the ICH Guidelines. But, no significant change was observed.

In wet granulation method the mass became very sticky and in dry granulation the mass became too much hard difficult for disintegrating the tablet.

In herbal tablets more common problems occurs due to the hygroscopic nature of the extract. To overcome this problem we have selected adsorbent material like stylod. Basically stylod is silica. Syloid is having good adsorbent property. Therefore it is widely used in formulating herbal tablets. These prepared tablets having good quality in terms of physical appearance and also passed the parameters which are used for the evaluation of tablets. In future these prepared tablets can be used modify with the film coating or to mask the taste can be sugar coating. To justify the shelf life we had did the three month stability study at room temperature and found no significance change in the quality of the tablets.

As we know the major issues faced in the herbal formulation is the quality of formulation and its shelf life. For that purpose suitable analytical methods can be used. That can do the quantitative evaluation of herbs and their formulation with respect to their marker compound. There are two types of marker compound are present in herbs. One is analytical marker and another is biological marker. Analytical markers are generally used for the standardization of herbs, extract or herbal formulation. And biological markers are used to assess the different bioactivity.

To justify this in this present work we had develop the analytical methods by using modern methods like HPLC and HPTLC.

All the methods were validated with respect to parameters including Linearity, Precision, Accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ), System suitability and Robustness.

HPLC method was used for simultaneous estimation of gallicacid and Oleanolic acid. Following are the parameters were used.

Column was Hyperchrom ODS BP C18 (Size: 250*4.6 mm,5µ). Flow rate was 1.0 ml/min. Detection wavelength was 222 nm. Mobile Phase was Ortho Phosphoric acid 0.1 % in Water: Methanol (5:95) It was filtered through 0.45 µm Nylon filter and sonicated for 5 min. Injection Volume was 20 µl through rheodyne manual injector. Temperature was Ambient. Retention Time was 2.8 min for Gallic acid and 9.9 min for Oleanolicacid.

HPTLC method was used for Simultaneous estimation of Gallicacid, Oleanolicacid And E-Guggulsterone

HPTLC Plate Aluminium plates pre coated with silica gel 60 F 254 (10 x 10 cm) was used. .The plates were activated at 110 0 C for 30 minutes Prior to chromatography. Development chamber Camag twin through glass chamber (20x20 cm) was used. The optimized chamber saturation time for mobile phase was 45 Min at room temp. Mobile Phase Toluene: Ethyl Acetate: Formic acid (7:6:1) Injection volume was 20 µl. Detection wavelength was UV at 270 nm and 368 nm after spraying with Methanolic Sulphuric acid.

In silico approach was used to check the anti-obesity potential of different active markers in the extract used in the tablet formulation.

Different target receptor was selected to check the binding affinity of the active markers to predict the anti-obesity activity.

The PDB file (1HLG) of receptor was downloaded from the PDB databank and ligands were downloaded from the ZINC database. Autodock Vina software was used to predict the binding interactions of markers and standard drugs with protein.

Following proteins ware selected for *in silico* approach

Pancreatic α-Amylase, Pancreatic Lipase, PPARs (peroxisome proliferator activated receptor) (PPARalpha), Leptin, Cannabinoid receptor type 1(CB1) and HMG CoA Reductase

Oleanolic acid bound efficiently to the active site of alpha-amylase with good complementarity, and the docking score is -9.0 kcal/mol. which is far more compared with standard drug acarbose (docking score -7.6 kcal/mol). These binding interactions present a clear view that Oleanolic acid can have good interactions with alpha-amylase.

Garcinia showed some interactions with human gastric lipase protein through hydrogen bonding and other nonbonding interactions but the interactions are not strong enough as the docking score is -4.7 kcal/mol while Oleanolic acid shoed good binding interaction with docking score of -8.4 kcal/mol. These binding interactions present a clear view that Oleanolic acid interact with human gastric lipase stronger then Garcinia and Orlistat.

Guggulsterone, Oleanolic acid, Mahanine, and Quercetin shown good binding interactions with the human PPAR alpha efficiently as the docking score is -7.9, -8.0, -8.5, and -9.5 kcal/mol, respectively. The reported drug Clofibrate has showed docking score of only -6.2 kcal/mol indicating poor interactions compared to marker compounds.

Mahanine and Oleanolic acid are showing interactions with the active site of human obesity protein, leptin with good complementarity, and the docking score is -6.4 and -7.9 kcal/mol, respectively for 1AX8 whereas -6.4 and -7.5 kcal/mol, respectively for 3V6O. The pose and

interaction suggested that the oleanolic acid binds strongly with the leptin in all the protein structures.

Quercetin, and oleanolic acid are bound efficiently to the active site of cannabinoid receptor type 1 with good complementarity, and the docking score is -8.1 and -8.2 kcal/mol, respectively. These binding interactions (Figure 7) present a clear view that quercetin, and oleanolic acid can irreversibly interacted cannabinoid receptor type 1. Binding interaction of quercetin, and oleanolic acid can irreversibly interact cannabinoid receptor type 1.

Quercetin, oleanolic acid, guggulsterone, and mahanine, are bound efficiently to the active site of human HMG-CoA reductase with good complementarity, and the docking score is -8.2, -8.9, -8.1 and -8.3 kcal/mol respectively. The standard drug atorvastatin formed many H-bonds but has less nonbonding interactions. All the interactions can be visualized.

From the above docking score and binding affinity towards the receptor it can be predicted the selected combination of markers (herbs) having anti-obesity potential.

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