

POLYCYSTIC OVARY SYNDROME

(Project-Report)

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Science degree in microbiology.

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of
MYTHREYAN.R(3830011) who carried out the project entitled “**POLYCYSTIC
OVARY SYNDROME**” under our supervision from October 2020 to March 2021.

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DATE:

A handwritten signature in blue ink, reading "R. Mythreyan". The signature is written in a cursive style with a large initial "R".

PLACE: SIGNATURE OF THE CANDIDATE

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine disturbance affecting women between 15 to 40 years of age. It is characterized by anovulation, infertility, and hyperandrogenism, with clinical manifestations of irregular menstrual cycles, hirsutism and acne. In addition, obesity and insulin resistance are common, along with an increased risk of diabetes, metabolic syndrome and other cardiovascular risk factors. Drugs such as metformin are used in the clinical side to treat PCOS. But the drug is not approved by the U.S Food and Drug Administration (FDA) for this use. Plant derived natural compounds used in our traditional Indian medicine have been showing better efficacy in the treatment of PCOS. The primary medicine for PCOS followed in siddha treatment is the bark of ashoka tree given in a powdered form. The presence of catechin, kaempferol, leucopelargonidin, epicatechin and leucocyanidin were found in the bark and flowers of ashoka tree. The two target proteins taken were follistatin and activin. Docking is done to prove the efficacy of these Indian medicines against PCOS. The most popular software used to predict protein-ligand docking is Accelrys Discovery Studio. The best docking score was noted for 5 structures against 2 target proteins-activin and follistatin. Out of the 5 drugs epicatechin gave the highest docking score against activin and leucocyanidin gave the highest docking score against follistatin for polycystic ovarian syndrome. The ADMET properties of these drugs were also verified.

Keywords: Polycystic ovarian syndrome, In-Silico approach, Indian medicine

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INTRODUCTION

PCOS

Polycystic ovarian syndrome is a condition where a hormonal imbalance affects follicular growth during the ovarian cycle causing the affected follicles to remain in the ovary. The retained follicle forms into a cyst & with each ovarian cycle a new cyst is formed leading to multiple ovarian cysts. Polycystic ovary syndrome is characterised by anovulation, infertility, and hyperandrogenism, with clinical manifestations of irregular menstrual cycles, hirsutism, and acne. The condition affects an estimated 5-10% of women of reproductive age, although this varies depending on the diagnostic criteria used. One of the commonest presenting complaints of women with polycystic ovary syndrome is anovulatory infertility. They also have increased prevalence of cardiovascular risk factors similar to that seen in the metabolic syndrome (which, like polycystic ovary syndrome, is characterised by insulin resistance). By the age of 40, up to 40% will have type 2 diabetes or impaired glucose tolerance. Polycystic ovary syndrome is therefore an important health concern and may represent a major health issue affecting young women today.

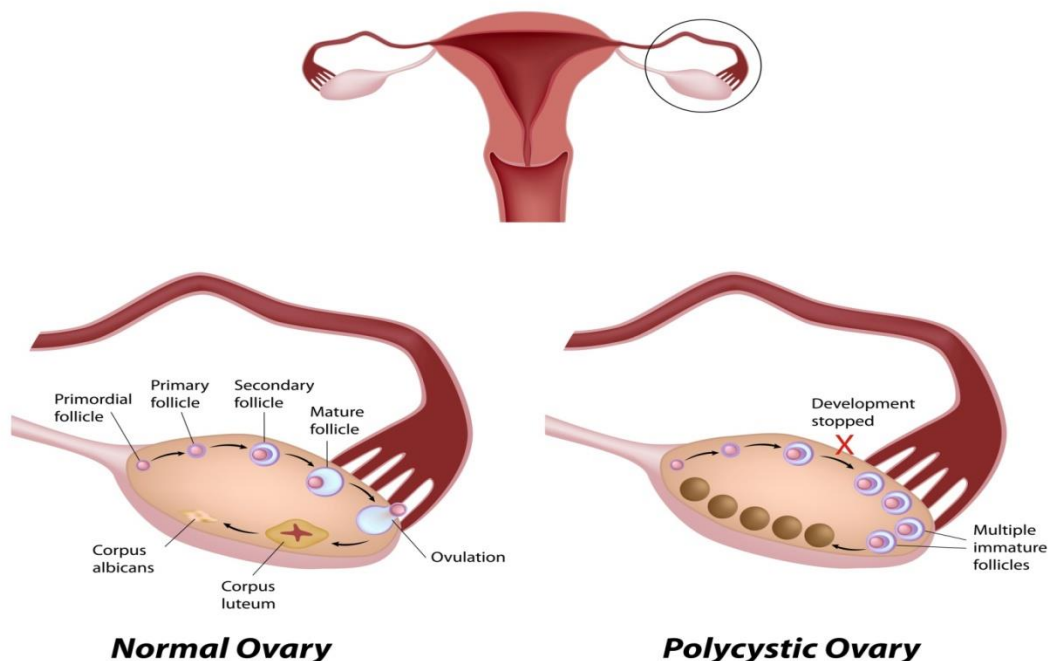


Fig (1): Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) affects 7-10% of reproductive age women, making it the most common endocrinopathy in this age group. Irregular menstrual cycles, hyperandrogenism and polycystic ovarian morphology are cardinal features of the syndrome. In addition, obesity and insulin resistance are common, along with an increased risk of diabetes, metabolic syndrome and other cardiovascular risk factors.

Polycystic ovarian syndrome is a most prevalent endocrinopathy. Incidence of this disease is increasing now a days because of sedentary lifestyles, pollution, excessive intake of junk food. It is obviously observed in women seeking medical advice for infertility as well as irregular menstrual bleeding. Majority of these cases could be treated either by hormonal therapy or surgical intervention. Ultrasonic morphologic shows evidence of ≥ 12 follicles measuring 2.9mm diameter in single plane during quiescent phase of ovary i.e. 2.7 days of cycle associated with obesity, oligomenorrhea, anovulation & hyperandrogenism, hyperinsulinemia- increased level of insulin in the blood is due to sensitivity of cells to insulin, a factor present in the blood stimulates androgen secretion by the ovarian stroma, the connective tissue of ovarian tissue of ovary & reduces serum sex hormone binding globin(SHBG) causing increased levels of free testosterone. Due to the presence of increased androgen in the ovary , the follicle undergoing maturation in the ovarian cycle is affected causing anovulation of particular follicle.

Modern medicine has been able to pinpoint a number of important factors indicating the disease determinants however the exact cause of the disease is unknown. Women suffering from PCOS often present with other associated symptoms including hirsutism – excessive body hair, menstrual disturbances, acne vulgaris and obesity. Ayurveda observes the disease looking for indications of the dosha responsible for the disorder. To fully understand the dosha responsible we will continue to look at what modern medicine has discovered about PCOS.

One of the key factors now being realized in modern medicine in relation to PCOS is hyperinsulinemia – increased levels of insulin in the blood. This indicates a direct link between obesity and PCOS as an increased level of insulin in the blood is due to de-sensitivity of cells to insulin, a factor present in diabetes mellitus type II. Increased insulin in the blood stimulates androgen secretion by the ovarian stroma –the connective tissue of the ovary and reduces serum sex hormone-binding globin (SHBG) causing increased levels of free testosterone. Due to the presence of increased androgen in the ovary, the follicle undergoing maturation in the ovary cycle

is affected causing anovulation of that particular follicle. The presence of insulin also impacts apoptosis – natural occurring death of a defective cell; of the follicle causing the follicle to continue to survive whereas under normal circumstance it would have perished. The ovarian cycle is governed by a hormonal feedback system moderated by the hypothalamus thus it requires constant feedback of hormonal levels for it to properly regulate the release of follicle stimulating hormone (FSH) and lutenizing hormone (LH) from the anterior pituitary gland. Both of these hormones play crucial parts in the development of the follicle during the ovarian cycle. As the hormonal levels in the body are imbalanced and the ovum is not released by the ovary the positive feedback mechanism to the hypothalamus to suppress FSH and LH release from the pituitary gland is impeded. However the hypothalamus is receiving positive and negative feedback from hormones but not due to the normal occurrence of the ovarian cycle. The hormonal feedback that the hypothalamus is receiving is due to high levels of estrogen that has been formed from free androgens in the peripheral tissue.

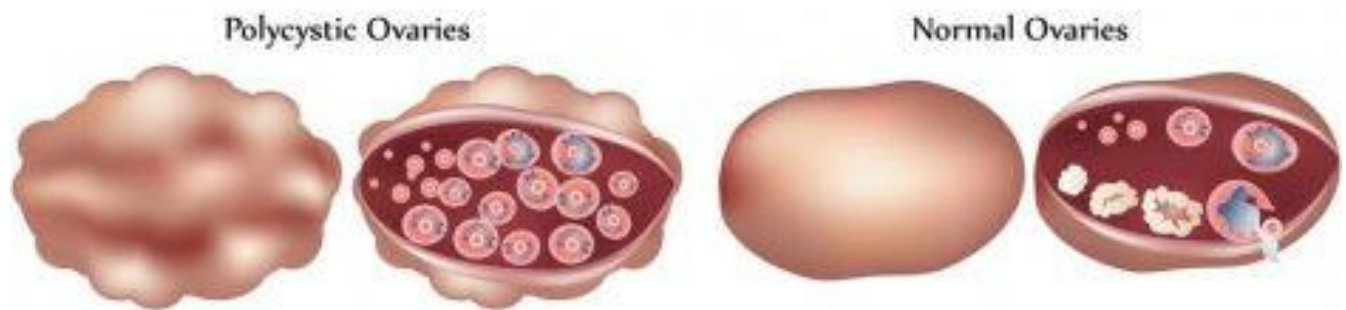


Fig (2): Ovaries affected by PCOS

FOLLISTATIN

Follistatin also known as activin-binding protein is a protein that in humans is encoded by the *FST* gene. Follistatin is an autocrine glycoprotein that is expressed in nearly all tissues of higher animals. It is a single chain glycosylated polypeptide that can bind to activin. When follistatin binds to activin it suppresses the role of activin to stimulate the secretion of Follicle Stimulating Hormone (FSH). FSH plays an important role in folliculogenesis and decrease in FSH level may arrest follicular development.

Its primary function is the binding and bionutralization of members of the TGF- β superfamily, with a particular focus on activin, a paracrine hormone.

Follistatin is a single-chain gonadal protein that specifically inhibits follicle-stimulating hormone release. The single *FST* gene encodes two isoforms, FST317 and FST344 containing 317 and 344 amino acids respectively, resulting from alternative splicing of the precursor mRNA. In a study in which 37 candidate genes were tested for linkage and association with polycystic ovary syndrome (PCOS) or hyperandrogenemia in 150 families, evidence was found for linkage between PCOS and follistatin.

ACTIVIN

Identified in 1986, activin is a protein complex that enhances FSH biosynthesis and secretion, and participates in the regulation of the menstrual cycle. Many other functions have been found to be exerted by activin, including roles in cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair, and endocrine function. Conversely inhibin down regulates FSH synthesis and inhibits FSH secretion.

Activin is a dimer composed of two identical or very similar beta subunits. Inhibin is also a dimer wherein the first component is a beta subunit similar or identical to the beta subunit in activin. However, in contrast to activin, the second component of the inhibin dimer is a more distantly-related alpha subunit. Activin, inhibin and a number of other structurally related proteins such as anti-Müllerian hormone, bone morphogenetic protein, and growth differentiation factor belong to the TGF- β protein superfamily. In the ovarian follicle, activin increases FSH

binding and FSH-induced aromatization. It participates in androgen synthesis enhancing LH action in the ovary and testis. In the male, activin enhances spermatogenesis.

ASHOKA TREE

Ashoka is the most ancient tree of India, generally known as a “ashok briksh”, botanist known as a *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging family *Caesalpinaceae*. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. *Saraca asoca* is reported to contain glycoside, flavanoids, tannins and saponins. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer. Antimenorrhagic Activity *Saraca asoca* dried bark has been used for menorrhagia in India. In India *Saraca asoca* dried bark as well as flower is given as a tonic to ladies in case of uterine disorders. *Saraca asoca* stem bark also used to treat all disorder associated with the menstrual cycle. *Saraca asoca* bark in Sri Lanka is used for menstrual disorder and menorrhagia. *Saraca asoca* bark in India, used as a uterine sedative and hot water extracts administered to human adult female stimulates the uterus similar to ergot, but without producing tonic contraction. Also employed in menorrhagia, as an emmenagogue, uterine sedative, uterine affections as well as used in several preparations related to female troubles. The drug is reported to stimulate the uterus, making the contraction more frequent and prolonged. The crystalline glycoside substance is also reported to stimulate uterine contraction. The phytochemical study shows the presence of epicatechin, kaempferol, leucopelargonidin, leucocyanidin and catechin in bark and flower of ashoka tree.

LITERATURE REVIEW

Familial polycystic ovarian syndrome (PCOS) has been proposed to be linked to a site near the follistatin gene. We studied the concentrations of circulating follistatin, activin A and inhibin B in well-characterized subjects with PCOS (n = 108) and controls without PCOS (n = 20). Mean (+/- SEM) concentrations of follistatin were higher ($P < 0.05$) in PCOS (0.27 +/- 0.03 ng/ml) than controls (0.15 +/- 0.02 ng/ml) and activin A were lower ($P < 0.05$) in PCOS (0.20 +/- 0.01ng/ml) than controls (0.24 +/- 0.02 ng/ml). Inhibin B concentrations were not different between the two groups: PCOS (0.06 +/- 0.01ng/ml), and controls (0.06 +/- 0.01ng/ml). It is proposed that higher concentrations of follistatin with lower concentrations of activin A may relate to follicular development not proceeding beyond 8-10 mm and may be partly responsible for the lack of pre-ovular follicle development in PCOS. Norman *et al*, 2001 Apr; 16(4):668-72

The candidate gene approach has already paid some dividends in trying to understand the complex genetics of polycystic ovary syndrome (PCOS). In terms of steroidogenic abnormalities, CYP11a-encoding P450 side chain cleavage-appears to be a major susceptibility locus. In relation to the well-described metabolic disturbances in PCOS, the insulin gene variable number tandem repeat (VNTR) appears to be a promising candidate, at least in populations studied in the UK. Finally, genes implicated in ovarian follicular development may have a role in the aetiology of PCOS, as demonstrated by recent identification of the follistatin gene as a potential disease locus. It seems unlikely that PCOS can be explained on the basis of a single gene disorder although, in a given family, one gene may have a predominant effect. An oligogenic model seems the most appropriate basis on which to understand the genetic origins of this very common disorder. The candidate gene approach has been useful to date, but it may prove important in the near future to perform an anonymous genome-wide scan to identify hitherto unheralded susceptibility loci. Franks S, Gharani N, McCarthy M, 2001 Jul-Aug;7(4):405-10

Polycystic ovary syndrome is characterised by anovulation, infertility, and hyperandrogenism, with clinical manifestations of irregular menstrual cycles, hirsutism, and acne. The condition affects an estimated 5-10% of women of reproductive age, although this varies depending on the

diagnostic criteria used. One of the commonest presenting complaints of women with polycystic ovary syndrome is anovulatory infertility. They also have increased prevalence of cardiovascular risk factors similar to that seen in the metabolic syndrome (which, like polycystic ovary syndrome, is characterised by insulin resistance). By the age of 40, up to 40% will have type 2 diabetes or impaired glucose tolerance. The use of metformin for women with polycystic ovary syndrome has aroused a tremendous amount of interest. Jonathan M Lord *et al*, 2003

Previous studies suggest that inhibin subunit expression is decreased in granulosa cells of women with polycystic ovary syndrome (PCOS). Inhibin A (220 +/- 38 vs. 400 +/- 72 IU/ml; $P < 0.05$) and inhibin B (75.4 +/- 10.4 vs. 139 +/- 26 ng/ml; $P < 0.05$) concentrations were lower in the follicular fluid of PCOS follicles compared with those of size-matched follicles from regularly cycling women. Inhibin A was also lower in the follicular fluid of PCOS compared with subordinate follicles from normal women (577 +/- 166 IU/ml; $P < 0.05$). Inhibin A concentrations increased with increasing follicle size, resulting in significantly higher follicular fluid concentrations in dominant follicles from normal women compared with PCOS follicles (2298 +/- 228 IU/ml; $P < 0.05$). These data demonstrate that inhibin A and inhibin B concentrations are significantly reduced in the follicular fluid of women with PCOS compared with those in the follicular fluid of size-matched follicles from normal women, consistent with the decreased inhibin subunit mRNA expression in previous studies. These findings point to the potential importance of inhibins in normal follicle development and suggest that inhibin deficiency may play a role in the follicle arrest associated with PCOS. Welt *et al*, 2005 Oct;90(10):5582-7. Epub 2005 Jul 19

Polycystic ovary syndrome is the commonest cause of anovulatory infertility and is managed with numerous drugs.¹ Before starting drug treatment, women should be helped to optimise their body weight to improve the chance of spontaneous ovulation and natural conception, improve the chance of successful response to ovulation induction drugs, and maximise their health in pregnancy. Extremes of opinion on weight loss have been eloquently outlined: Balen and colleagues suggested that drug treatment should be withheld from women with a body mass index greater than 35 owing to the dangers of pregnancy in obese women,² and Laredo emphasized the concern of stigmatizing overweight women by such withholding of fertility treatment.³ Options for drug treatment may seem more straightforward, but a multicentre placebo controlled trial of

metformin reported in this week's *BMJ* by Moll and colleagues (p 1485) challenges that view. Hesham Al-Inany, assistant professor (kaainih@link.net), Neil Johnson, associate professor, 22 June 2006

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of the women of reproductive age. Familial clustering of PCOS has been consistently reported suggesting that genetic factors play a role in the development of the syndrome although PCOS cases do not exhibit a clear pattern of Mendelian inheritance. It is now well established that PCOS represents a complex trait similar to type-2 diabetes and obesity, and that both inherited and environmental factors contribute to the PCOS pathogenesis. A large number of functional candidate genes have been tested for association or linkage with PCOS phenotypes with more negative than positive findings. Lack of universally accepted diagnostic criteria, difficulties in the assignment of male phenotype, obscurity in the mode of inheritance, and particularly small sample size of the study populations appear to be major limitations for the genetic studies of PCOS. In the near future, utilizing the genome-wide scan approach and the HapMap project will provide a stronger potential for the genetic analysis of the syndrome. Ugur Unluturk *et al*, 2007 Feb 25. doi: 10.1155/2007/49109 PMID: PMC1820621

Follistatin has been reported as a candidate gene for polycystic ovary syndrome (PCOS) from linkage and association studies. Acting to regulate the development of ovarian follicles and as an antagonist to aromatase activity, alterations in follistatin function or expression may result in key features of PCOS such as reduced serum FSH, impaired ovarian follicle development and augmented ovarian androgen production. We investigated polymorphisms in the FST gene to determine if genetic variation is associated with susceptibility to PCOS or key phenotypic features of PCOS patients in a case-control association study. One hundred and seventy-three PCOS patients of Caucasian descent (mean age 30.0 +/- 4.8 years), conforming to the NIH diagnostic criteria, were recruited from a clinical practice database and 107 normal ovulating women (mean age 38.8 +/- 13.4 years) were recruited from the general community as control subjects. Morphometric data, biochemistry and genomic DNA were collected from study subjects and genotyping was performed on seven Single nucleotide polymorphisms (SNPs) in the FST gene region. Jones *et al*, 2007 Apr;13(4):237-41.

Women with PCOS had serum total inhibin levels twice as high as in the control group (median = 115 vs. 47 pg/mL). Dimeric inhibin A concentration was lower in the PCOS group compared with controls (10 vs. 25 pg/mL), whereas inhibin B concentration did not differ significantly between PCOS and control groups. There was no difference of either total inhibin or inhibin A levels between lean and overweight women with PCOS, whereas the inhibin B level was higher in the lean PCOS subgroup compared with the overweight PCOS subgroup. Total inhibin did not show any linear correlation with inhibin B, inhibin A, or serum gonadotropins. Women with PCOS have high serum concentration of total inhibin but not of inhibin A or inhibin B, thus suggesting that PCOS women have an impaired processing of alpha-inhibin precursor proteins. Tsigkou *et al*, 2008 Nov;90(5):1859-63. doi: 10.1016/j.fertnstert.2007.08.082

Ashoka is the most ancient tree of India, generally known as “ashok briksh”, botanist known as a *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging family Caesalpinaceae. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. *Saraca asoca* is reported to contain glycoside, flavanoids, tannins and saponins. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer. This review contains the Pharmacognostical account of various parts of plant, Phytochemical constituent and different reported pharmacological activity.

The Phytochemical study show in the bark of plant presence of (-) epicatechin, procyanidin p2,11'-deoxyprocyanidin B, (+) catechin, (24, E)- 24- methyl-cholesta-5-en-3p-ol (22 E, 21E)-24- ethycholesta-5,22 dien-33-ol,(24 E)-24- ethylcholesta-5-en-3-p-ol,leucopelargonidin-3-O-p-Dglucoside,leucopelargonidin and leucocyanidin.The flower part of plant contain Oleic, linoleic, palmitic and stearic acids,P-sitosterol, quercetin, kaempferol- 3-0-P-D- glucoside, quercetin- 3-0-P-D-glucoside, apigenin- 7-0-p-D-glucoside, pelargonidin- 3, 5- diglucoside, cyanidin-3, 5- diglucoside, palmitic, stearic, linolenic, linoleic, p and y sitosterols, leucocyanidin and gallic acid. Seed and Pod contains oleic, linoleic, palmitic and stearic acids, catechol, (-) epicatechol and leucocyanidin [2,10,11,12]. *Saraca asoca* stem bark also used to treat all disorder associated with

the menstrual cycle [26,27]. *Saraca asoca* bark in Sri Lanka used for menstrual disorder and menorrhagia [28,29]. *Saraca asoca* bark in India, used as a uterine sedative and hot water extracts administered to human adult female stimulates the uterus similar to ergot, but without producing tonic contraction. Also employed in menorrhagia, as an emmenagogue, uterine sedative, uterine affections as well as used in several preparations related to female troubles [30,31,32,33]. *Saraca indica* bark, in Pakistan, employed for uterine affection and menorrhagia. *Saraca indica*, in India, dried bark, used as an astringent in menorrhagia, to stop excessive uterine bleeding [34], also as a refrigent, demulcent, uterine disorders, regular menstrual pain in abdomen, used for uterine problems. P. Pradhan *et al* Journal of Chemical and Pharmaceutical Research, 2009, 1 (1):62-71

Polycystic ovary syndrome (PCOS) is very common endocrinological disorder in premenopausal women. Activin and inhibin are two closely related protein complexes that have almost directly opposite biological effects. Activin enhances FSH biosynthesis and secretion, and participates in the regulation of the menstrual cycle. Many other functions have been found to be exerted by activin, including roles in cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair and endocrine function. Activin and Inhibin are produced in the gonads, pituitary gland, placenta, and other organs. The results of the present study showed that serum Inhibin A level is low in PCOS, while no difference in the level of serum Activin A in PCOS patients in comparing with control group. The results of the present study showed that serum Inhibin A was negatively correlated with age, and Directly correlated with serum Testosterone and E2. Activin A level in PCOS women showed a significant Positive correlation with LH and LH/FSH ratio. Hussein Kadhem Al-Hakeim (PhD) * Maha Abdul Saheb Ridha, VOL.5 / NO.2 / year : 2013

Polycystic ovary syndrome (PCOS) affects 7-10% of reproductive age women, making it the most common endocrinopathy in this age group (1,2). Irregular menstrual cycles, hyperandrogenism and polycystic ovarian morphology are cardinal features of the syndrome (3). In addition, obesity and insulin resistance are common, along with an increased risk of diabetes, metabolic syndrome and other cardiovascular risk factors. Mitra Daneshrad L. Ac., March 2013

Follistatin-like protein 1 (Fstl1) is a secreted glycoprotein of the follistatin family. Fstl1 is secreted by C2C12 cells, and Akt1 over-expression in skeletal muscle leads to its induction in muscle and

increased circulating levels. So far, secretion of Fstl1 by human myotubes and the effect of exercise on its circulating levels have not been investigated. Here, we examined both the regulation of Fstl1 expression and secretion in primary human skeletal muscle cells and the effect of acute exercise on Fstl1 serum concentrations in humans. We show that human myotubes express and secrete Fstl1 in a differentiation-dependent manner. Furthermore, IFN γ and IL-1 β significantly increase Fstl1 secretion. Electrical pulse stimulation (EPS)-induced contractile activity of myotubes did not regulate Fstl1. Interestingly, we observed that 60 min cycling increased serum Fstl1 level by 22%. In conclusion, we demonstrate that Fstl1 is expressed and secreted by human myotubes and plasma Fstl1 levels are increased after exercise. Gorgens *et al*, 2013 May;119(2):75-8+0. doi: 10.3109/13813455.2013.768270

Polycystic ovarian syndrome (PCOS) also known by the name of stein-leventhal syndrome. Incidence of this condition is growing amongst young women in reproductive age. It is almost ranging between 5-10% of young women coming for infertility, it is now increasingly perceived as disorder of changed life styles and is a rainbow metabolic syndrome Research shows that history of gestational diabetes 40% are cases at PCOS. Death rates amongst PCOS due to diabetes mellitus and metabolic syndrome s 5:1.4 to normal population, excessive exposure to estrogen unopposed by progesterone could cause greater incidence of malignancies endometrium and breast. Infertility is by far the most common feature due to anovulation, therefore the present study was carried out for clinical evaluation of the efficacy of ayurveda treatment on PCOS. The treatment was conducted for duration of 3 months the response to the treatment was recorded by parameters, the result revealed that PCOS can be cured by successfully by using this ayurvedic regimen. Dr.Bhagyashri Mahavir Khot *et al*. Volume 3, Issue 4 (May 2013), Pp 21-25

Polycystic ovary syndrome (PCOS) is common and has reproductive and metabolic manifestations. Activin A and follistatin levels remain controversial and activin B levels are unstudied in PCOS. The aim of this study was to evaluate activin A, activin B and follistatin levels and to examine their associations with metabolic status in overweight and obese women with and without PCOS. Cross-sectional study assessing overweight and obese, premenopausal women with PCOS (n = 51, n = 26 National Institutes of Health (NIH) and n = 25 non-NIH) and without PCOS (n = 25 controls). Outcomes included activin A, activin B, follistatin and activin A/follistatin ratio and the association of the activins and follistatin with metabolic variables. Follistatin is elevated

and activins A and B are not different between PCOS and controls. Follistatin and activin A are related to metabolic parameters in women with and without PCOS. Follistatin may potentially act as a marker of or be involved in the pathophysiology of both reproductive and metabolic features of PCOS. Helena Teede *et al*, October 2013 Volume 62, Issue 10, Pages 1394–1400

Polycystic ovary syndrome (PCOS) is a heterogeneous female endocrinopathy which is characterized by chronic anovulation and clinical or biochemical Effects of OCPs and metformin on PCOS atherogenic risk 489 hyperandrogenemia as well as insulin resistance and a clustering of cardiovascular risk factors.¹ Even in the absence of detectable metabolic abnormalities, lean PCOS women have increased circulating levels of advanced glycated end products (AGEs), which are known proinflammatory and proatherogenic molecules. The diagnosis of PCOS was based on the presence of irregular menstrual cycles (eight or fewer menses per year) as well as elevated serum levels of testosterone and/or clinical symptoms of hyperandrogenism, according to the National Institute of Child Health and Human Development conference. Metformin, the most widely prescribed insulin sensitizer, appears to improve metabolic aspects and cardiovascular risk factors in women with PCOS. In particular, metformin treatment for 6 months was shown to alleviate the proinflammatory status and reduce serum AGEs levels in women with PCOS. Charikleia Christakou *et al* , HORMONES 2014, 13(4):488-497

MATERIALS AND METHODS

TARGET PREPARATION

PDB

The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organisations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB.

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (i.e., secondary) databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize structures based on genes.

The file format initially used by the PDB was called the PDB file format. This original format was restricted by the width of computer punch cards to 80 characters per line. Around 1996, the "macromolecular Crystallographic Information file" format, mmCIF, started to be phased in. An XML version of this format, called PDBML, was described in 2005. The structure files can be downloaded in any of these three formats. The structure files may be viewed using one of several open source computer programs, including Jmol, Pymol, and Rasmol. Some other free, but not open source programs include ICM-Browser, VMD, MDL Chime, UCSF Chimera, Swiss-PDB Viewer, StarBiochem (a Java-based interactive molecular viewer with integrated search of protein databank), Sirius, and VisProt3DS (a tool for Protein Visualization in 3D stereoscopic view in anaglyph and other modes). The RCSB PDB website contains an extensive list of both free and commercial molecule visualization programs and web browser plugins.

URL: <http://www.rcsb.org/pdb>

NCBI

The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper. The NCBI houses a series of databases relevant to biotechnology and biomedicine. Major databases include GenBank for DNA sequences and PubMed, a bibliographic database for the biomedical literature. Other databases include the NCBI Epigenomics database. All these databases are available online through the Entrez search engine. The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

NCBI is directed by David Lipman, one of the original authors of the BLAST sequence alignment program and a widely respected figure in bioinformatics. He also leads an intramural research program, including groups led by Stephen Altschul (another BLAST co-author), David Landsman, Eugene Koonin (a prolific author on comparative genomics), John Wilbur, Teresa Przytycka, and Zhiyong Lu.

URL: <http://www.ncbi.nlm.nih.gov>

SWISS-MODEL

SWISS-MODEL is a structural bioinformatics web-server dedicated to homology modeling of protein 3D structures. Homology modeling is currently the most accurate method to generate reliable three-dimensional protein structure models and is routinely used in many practical applications. Homology (or comparative) modelling methods make use of experimental protein structures ("templates") to build models for evolutionary related proteins ("targets").

Today, SWISS-MODEL consists of three tightly integrated components: The SWISS-MODEL pipeline - a suite of software tools and databases for automated protein structure modelling, The SWISS-MODEL Workspace - a web-based graphical user workbench, The SWISS-MODEL Repository - a continuously updated database of homology models for a set of model organism proteomes of high biomedical interest.

URL: <http://swissmodel.expasy.org/>

LIGAND PREPARATION

PUBMED

PubMed is a free search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval.

PubMed is a free resource that is developed and maintained by the National Center for Biotechnology Information (NCBI), at the U.S. National Library of Medicine (NLM), located at the National Institutes of Health (NIH).

Publishers of journals can submit their citations to NCBI and then provide access to the full-text of articles at journal web sites using LinkOut.

PUBCHEM

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PubChem database.

PubChem contains its own online molecule editor with SMILES/SMARTS and InChI support that allows the import and export of all common chemical file formats to search for structures and fragments.

Each hit provides information about synonyms, chemical properties, chemical structure including SMILES and InChI strings, bioactivity, and links to structurally related compounds and other NCBI databases like PubMed.

In the text search form the database fields can be searched by adding the field name in square brackets to the search term. A numeric range is represented by two numbers separated by a colon. The search terms and field names are case-insensitive. Parentheses and the logical operators AND, OR, and NOT can be used. AND is assumed if no operator is used.

DOCKING

ACCELRYS DISCOVERY STUDIO

Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys. The product suite has a strong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 and more.

Accelrys Discovery Studio® software provides comprehensive modeling and simulation capabilities for computational chemists, computational biologists, and other scientists engaged in small molecule and biotherapeutics-based research. The latest release is a comprehensive product update that extends our portfolio of small molecule drug design and market-leading biological simulation tools.

Discovery Studio provides software applications covering Simulations, Ligand Design, Pharmacophore modeling, Structure-based Design, Macromolecule design and validation, Macromolecule engineering, QSAR, ADME and Predictive toxicity.

TARGETED PROTEIN

FOLLISTATIN

Follistatin also known as activin-binding protein is a protein that in humans is encoded by the *FST* gene. Follistatin is an autocrine glycoprotein that is expressed in nearly all tissues of higher animals. It is a single chain glycosylated polypeptide that can bind to activin. When follistatin binds to activin it suppresses the role of activin to stimulate the secretion of Follicle Stimulating Hormone (FSH). FSH plays an important role in folliculogenesis and decrease in FSH level may arrest follicular development.

Its primary function is the binding and bionutralization of members of the TGF- β superfamily, with a particular focus on activin, a paracrine hormone.

Follistatin is a single-chain gonadal protein that specifically inhibits follicle-stimulating hormone release. The single *FST* gene encodes two isoforms, FST317 and FST344 containing 317 and 344 amino acids respectively, resulting from alternative splicing of the precursor mRNA. In a study in

which 37 candidate genes were tested for linkage and association with polycystic ovary syndrome (PCOS) or hyperandrogenemia in 150 families, evidence was found for linkage between PCOS and follistatin.

ACTIVIN

Activin is a dimer composed of two identical or very similar beta subunits. Inhibin is also a dimer wherein the first component is a beta subunit similar or identical to the beta subunit in activin. However, in contrast to activin, the second component of the inhibin dimer is a more distantly-related alpha subunit. Activin, inhibin and a number of other structurally related proteins such as anti-Müllerian hormone, bone morphogenetic protein, and growth differentiation factor belong to the TGF- β protein superfamily.

In the ovarian follicle, activin increases FSH binding and FSH-induced aromatization. It participates in androgen synthesis enhancing LH action in the ovary and testis. In the male, activin enhances spermatogenesis.

TARGETED NATURAL PRODUCTS

- catechin
- epicatechin
- kaempferol
- leucocyanidin
- leucopelargonidin

RESULTS

FOLLISTATIN

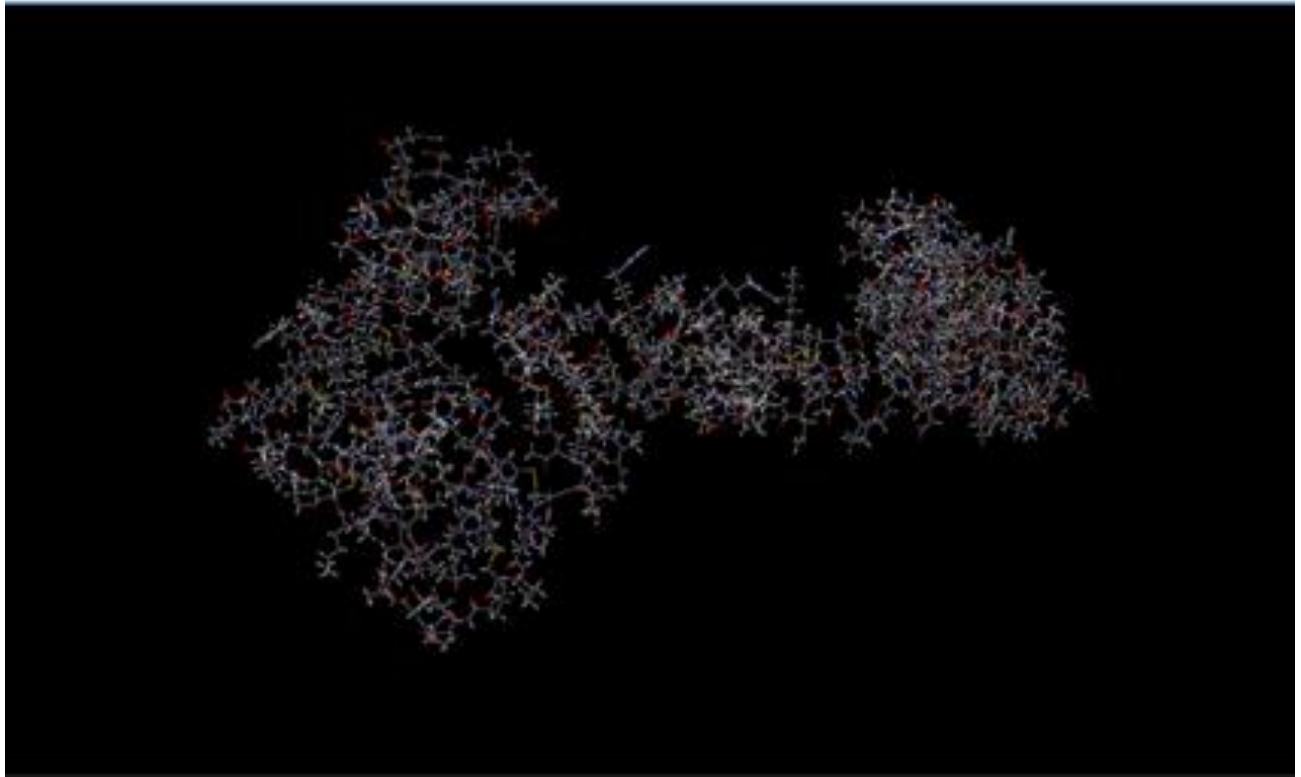


Fig (3): Structure of Follistatin

Follistatin also known as activin-binding protein is a protein that in humans is encoded by the *FST* gene. Follistatin is a single-chain gonadal protein that specifically inhibits follicle-stimulating hormone release. The single *FST* gene encodes two isoforms, FST317 and FST344 containing 317 and 344 amino acids respectively, resulting from alternative splicing of the precursor mRNA.

FOLLISTATIN AMINO ACID LABELS

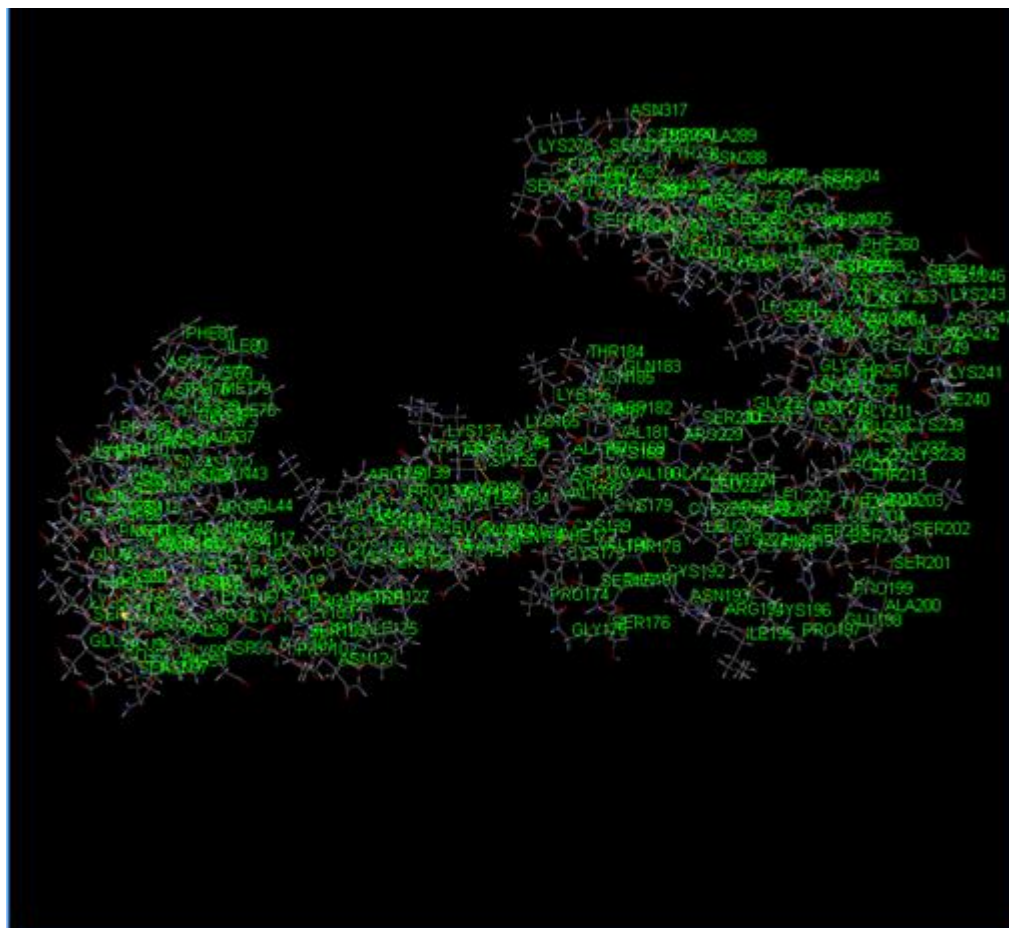


Fig (4):Follistatin amino acid labels

FOLLISTATIN BINDING SITES

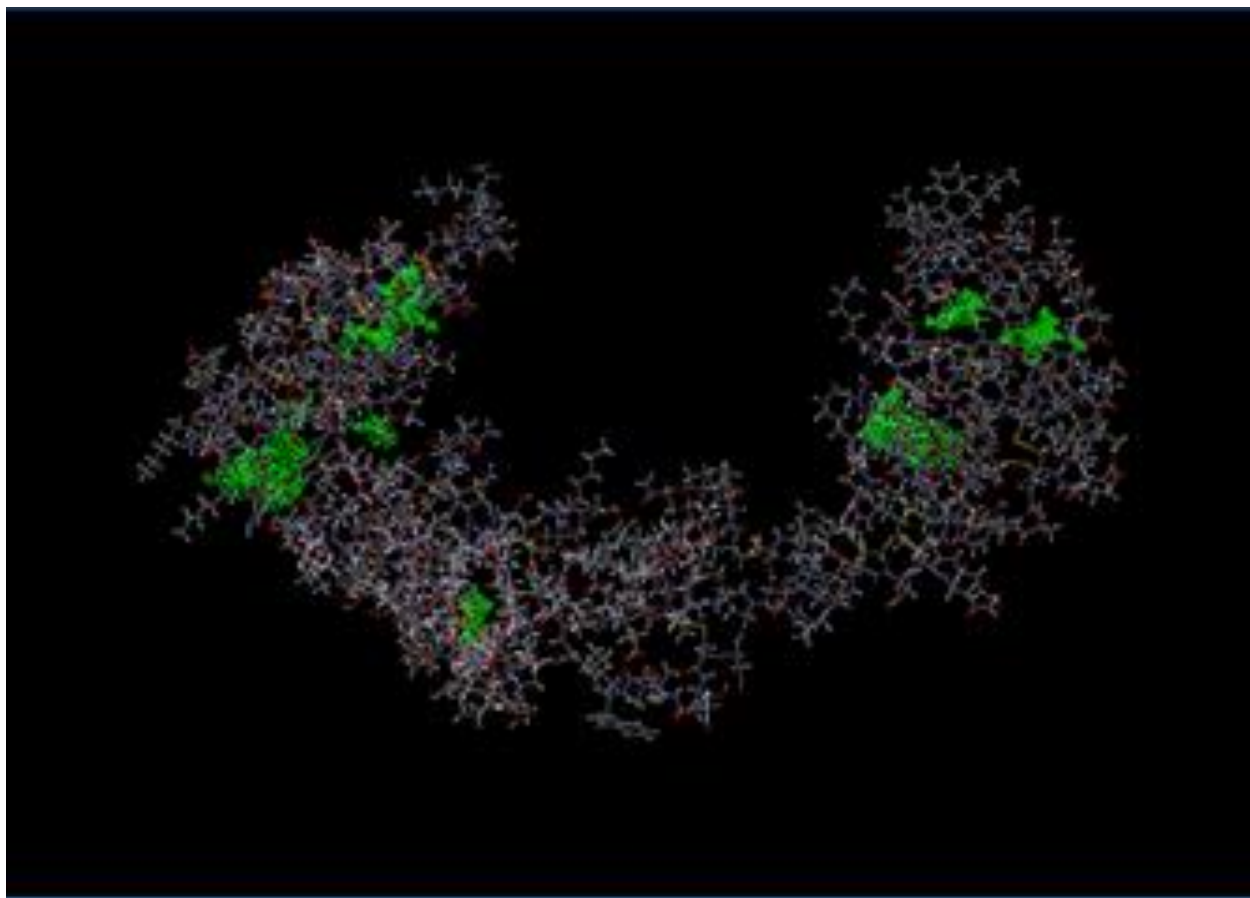


Fig (5): Follistatin binding sites

EPICATECHIN

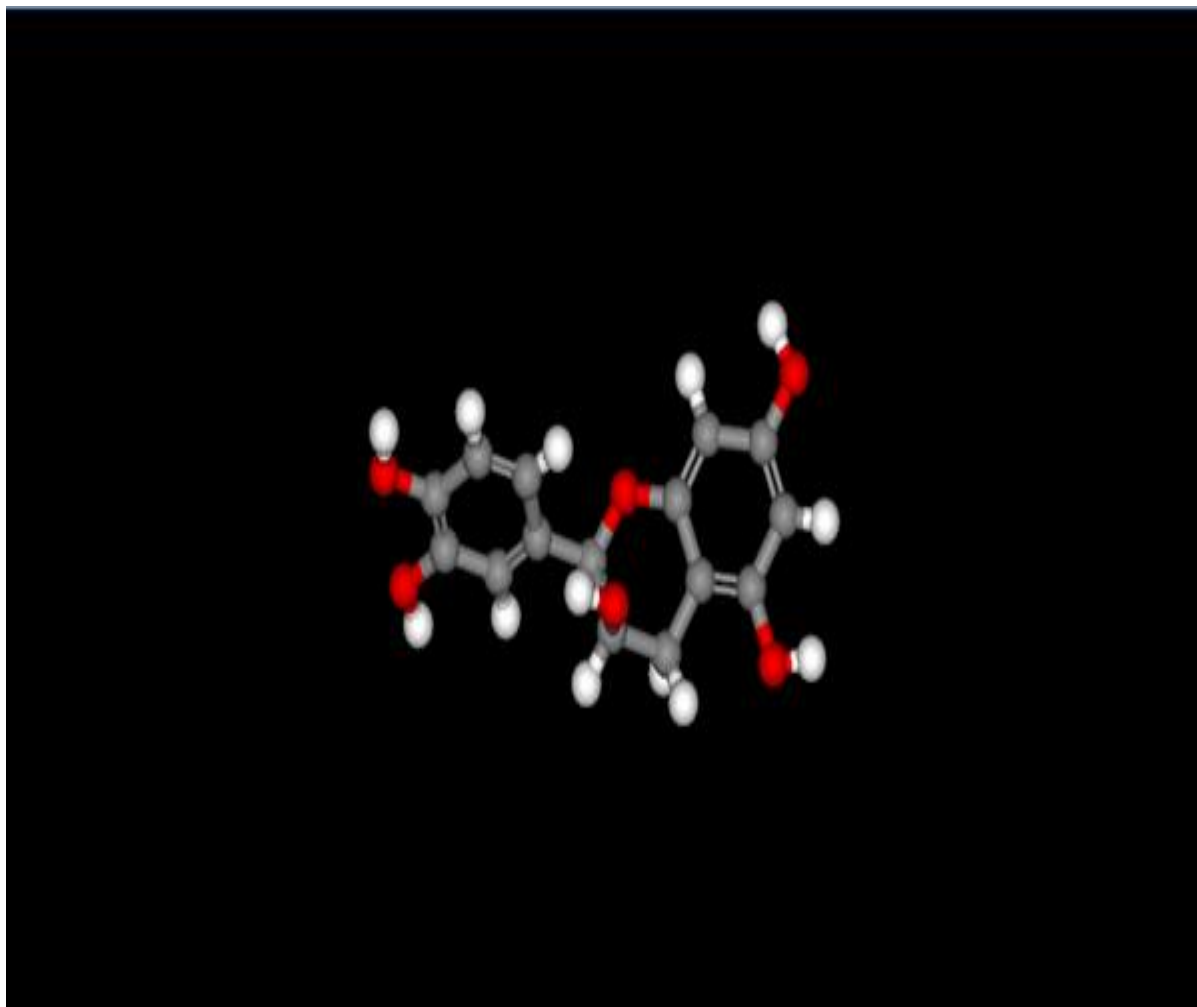


Fig (6): Structure of Epicatechin

FOLLISTATIN-EPICATECHIN DOCKING

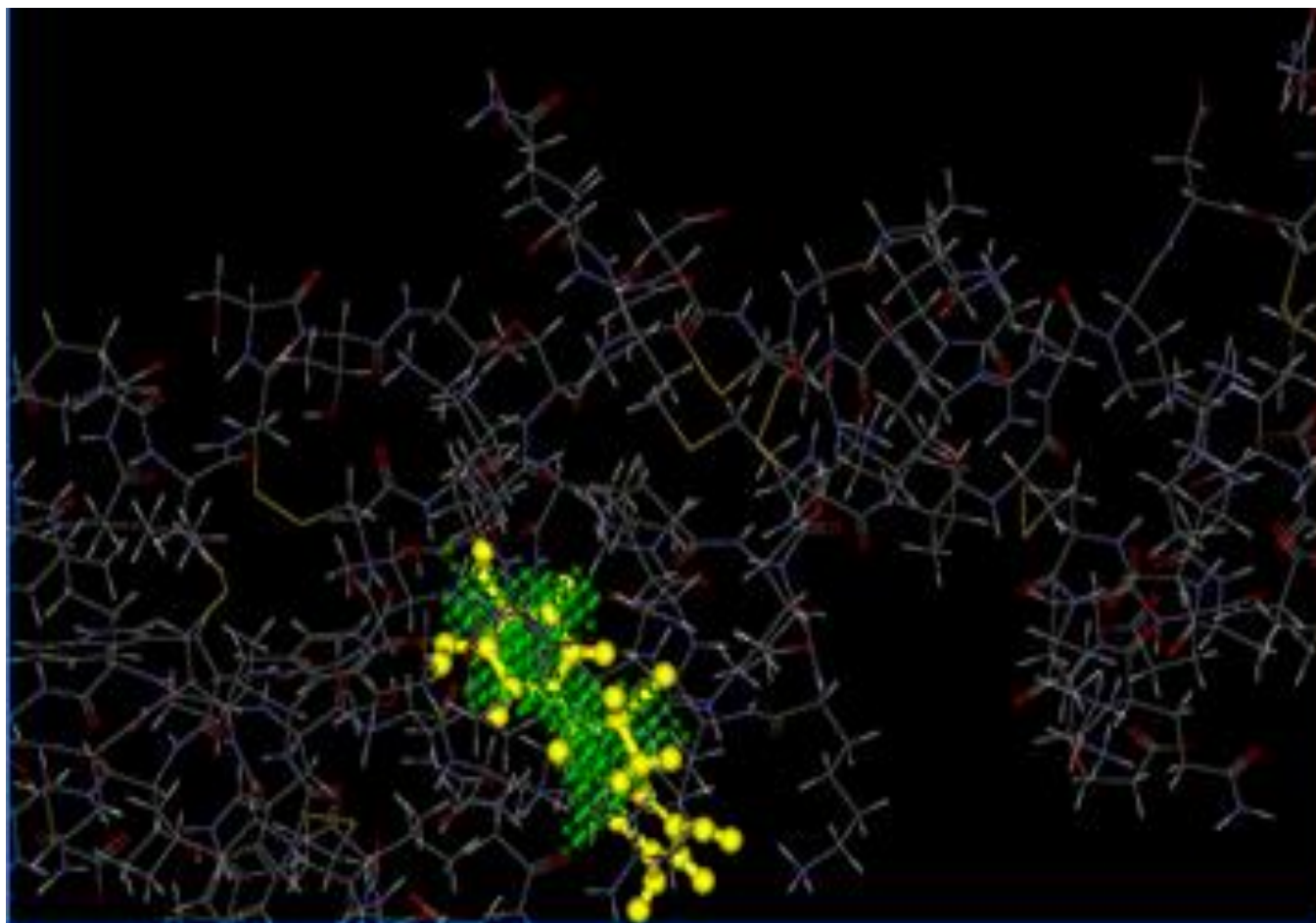


Fig (7): Interaction of Follistatin and Epicatechin

DOCK SCORE: 40.723

KAEMPFEROL

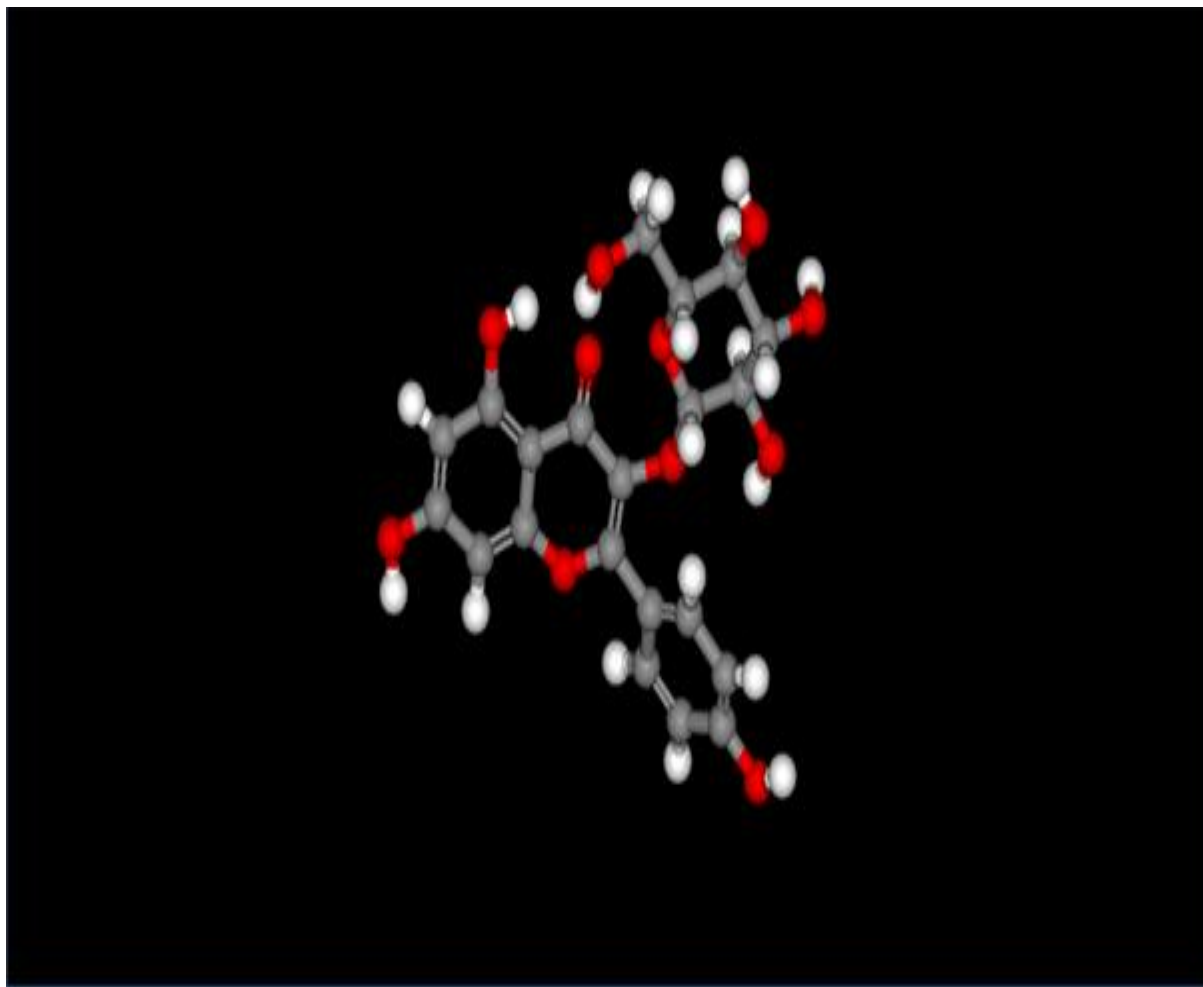


Fig (8): Structure of Kaempferol

FOLLISTATIN-KAEMPFEROL DOCKING

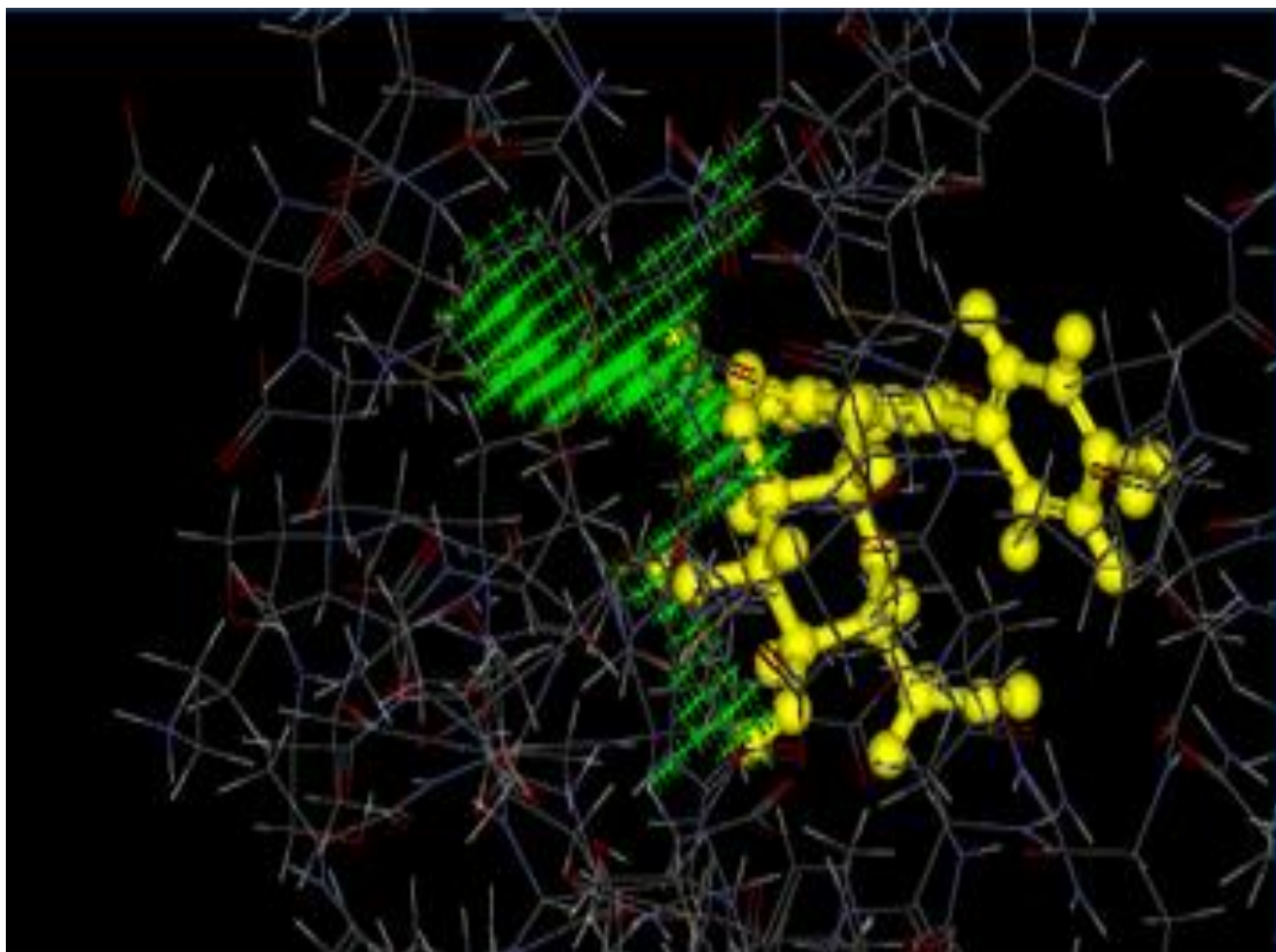


Fig (9):Interaction of Follistatin and Kaempferol

DOCKSCORE: 32.787

LEUCOPELARGONIDIN

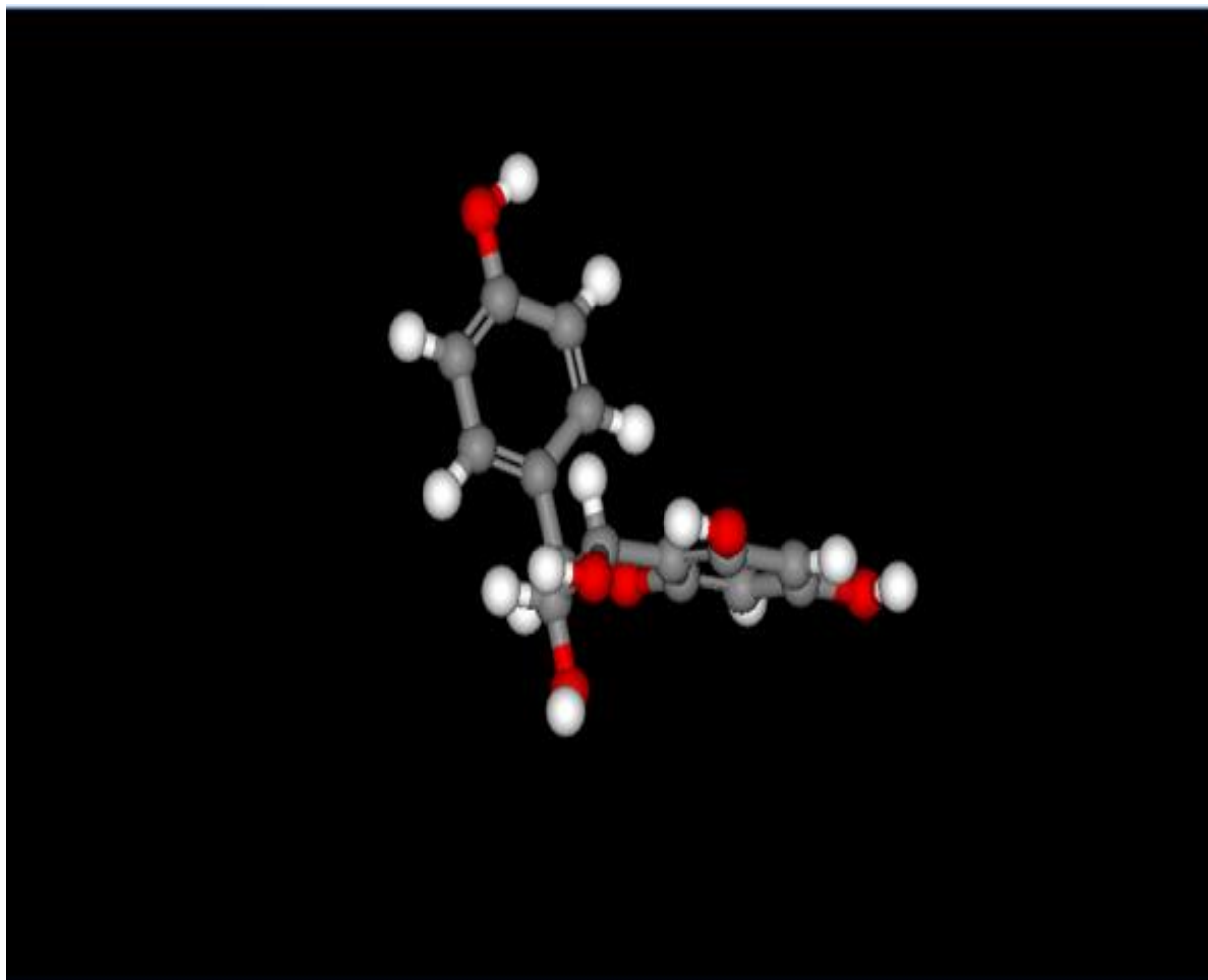


Fig (10):Structure of Leucopelargonidin

FOLLISTATIN-LEUCOPELARGONIDIN DOCKING

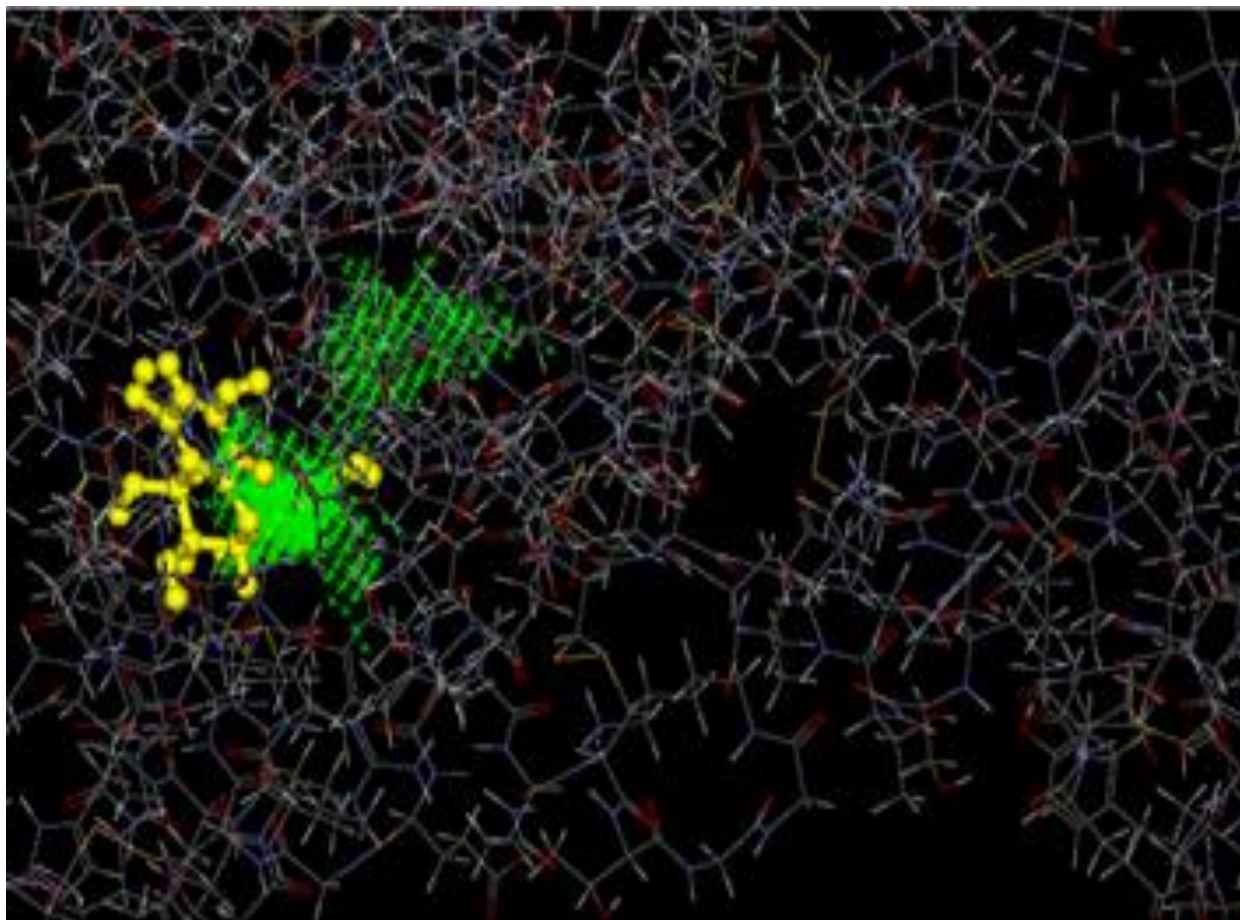


Fig (11):Interaction of Follistatin and Leucopelargonidin

DOCK SCORE: 38.501

LEUCOCYANIDIN

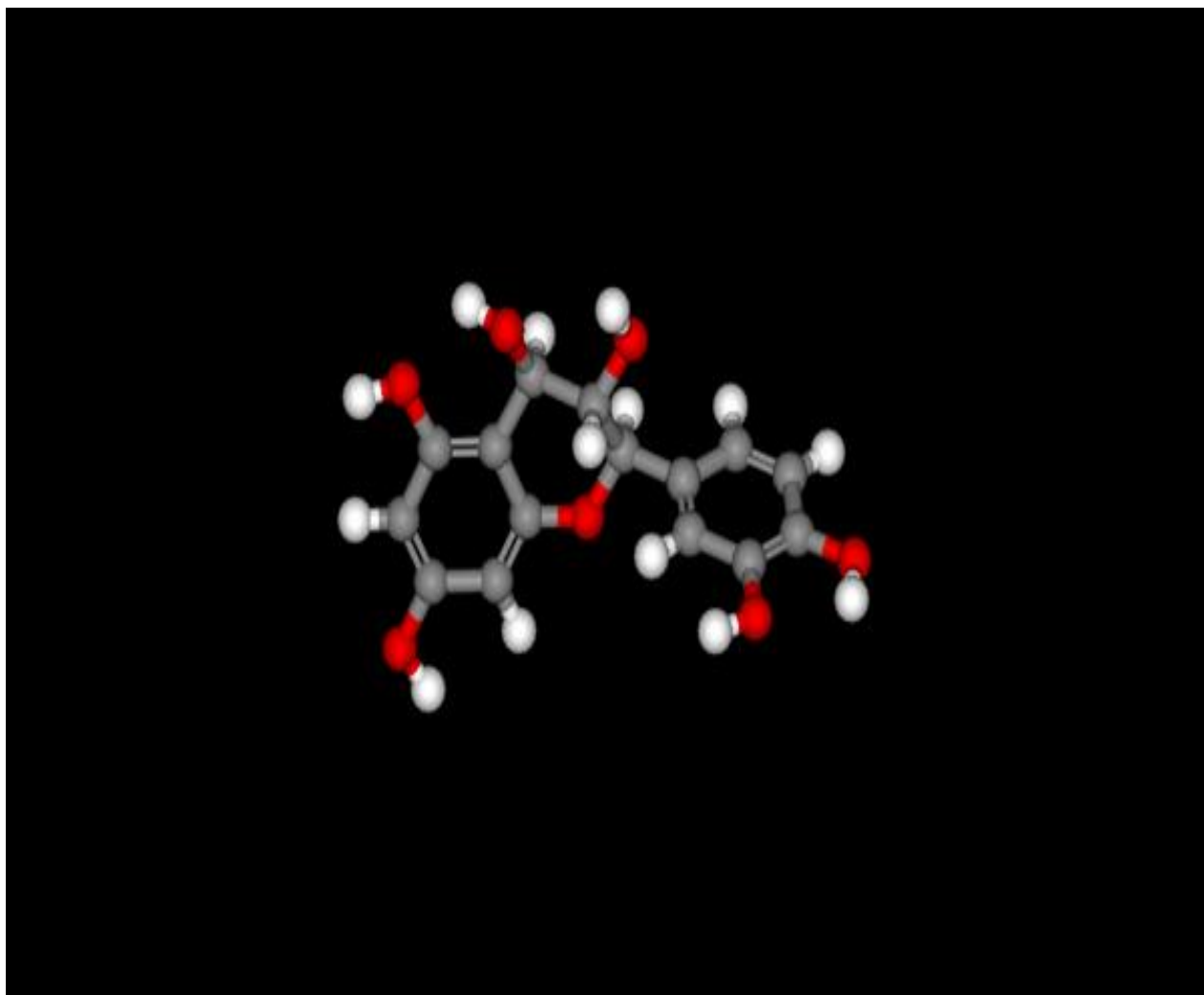


Fig (12):Structure of Leucocyanidin

FOLLISTATIN-LEUCOCYANIDIN DOCKING

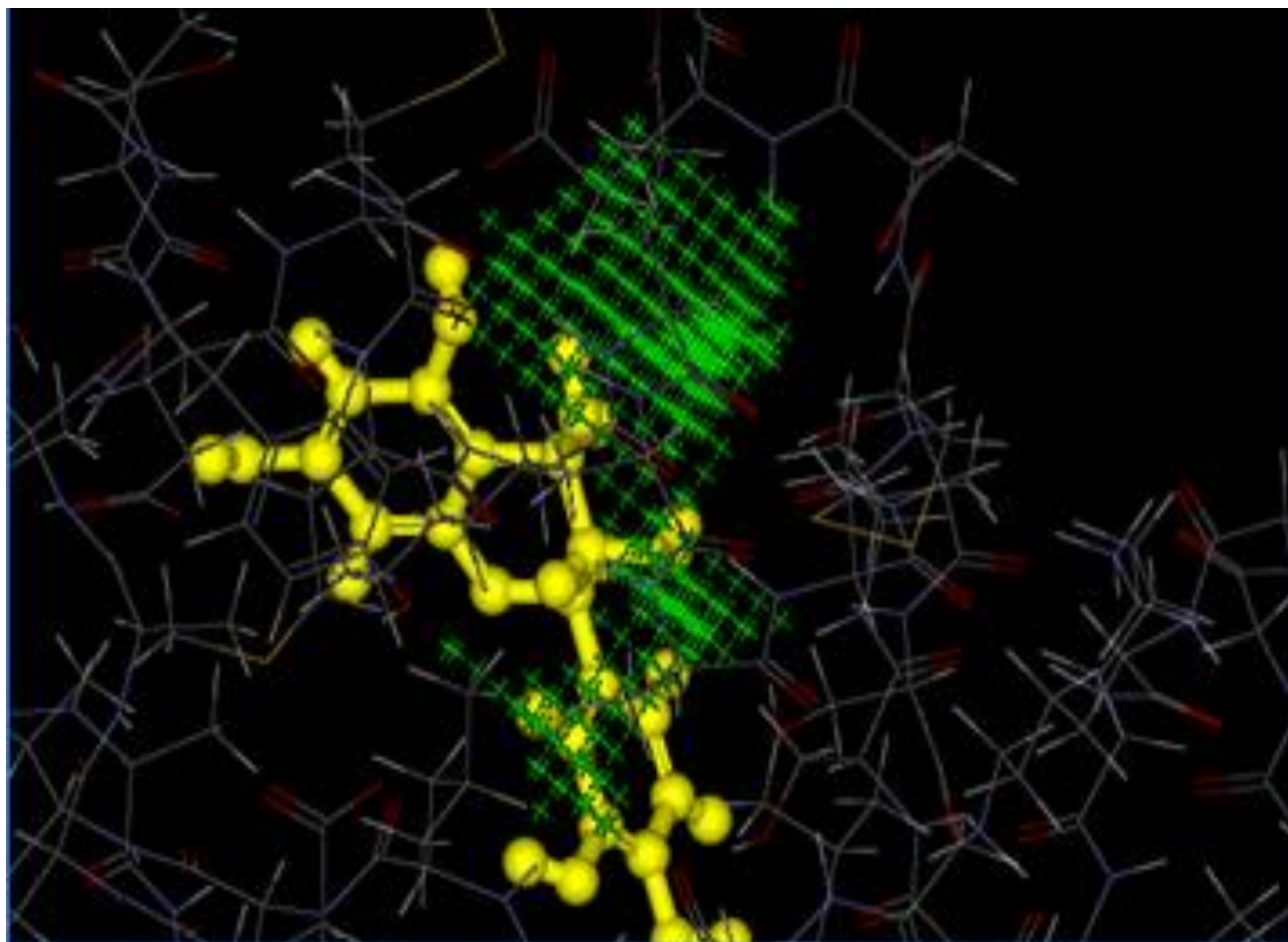


Fig (13):Interaction of Follistatin and Leucocyanidin

DOCK SCORE: **47.103**

CATECHIN

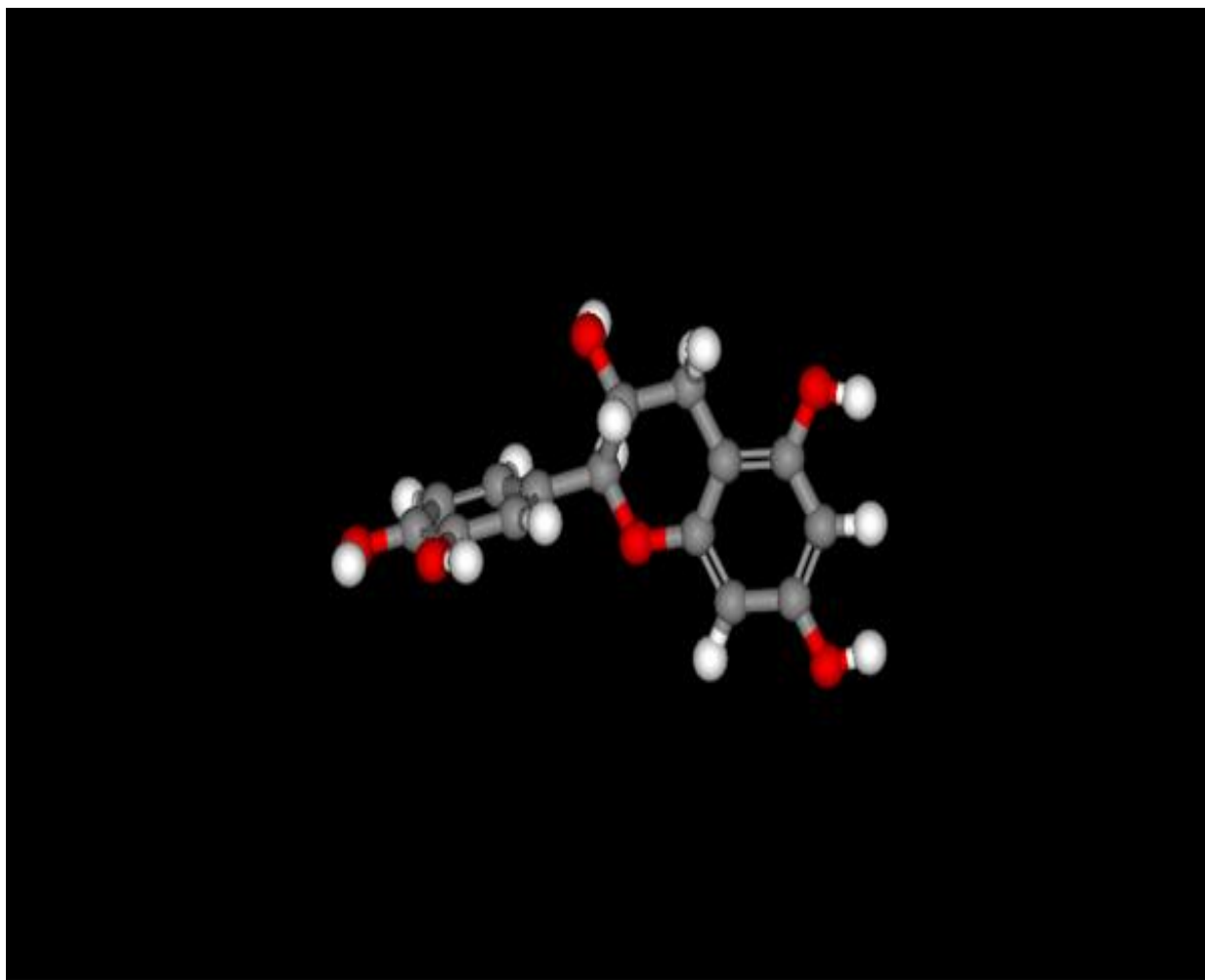


Fig (14): Structure of Catechin

FOLLISTATIN-CATECHIN DOCKING

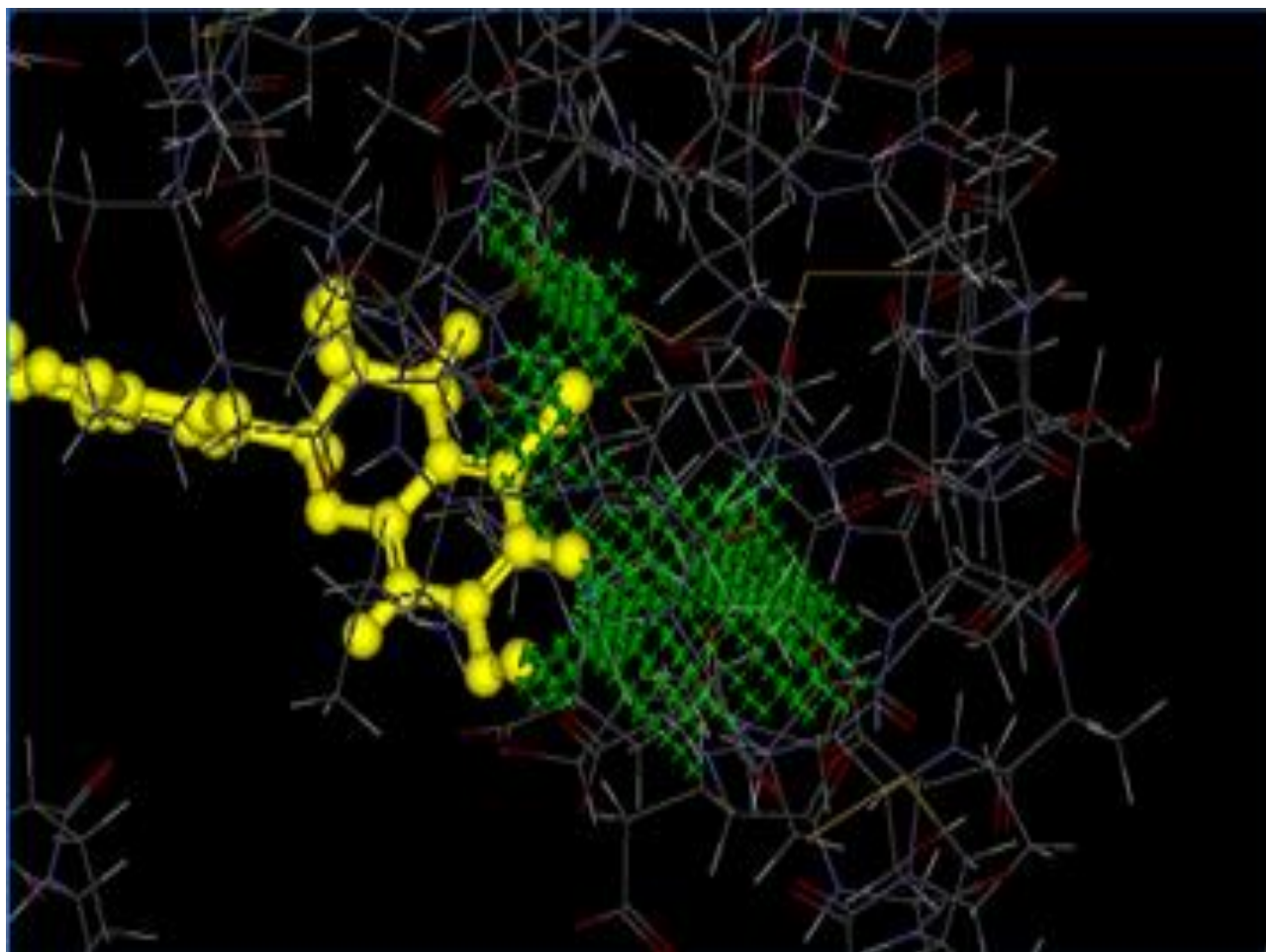


Fig (15):Interaction of Follistatin and Catechin

DOCK SCORE: 33.452

ACTIVIN

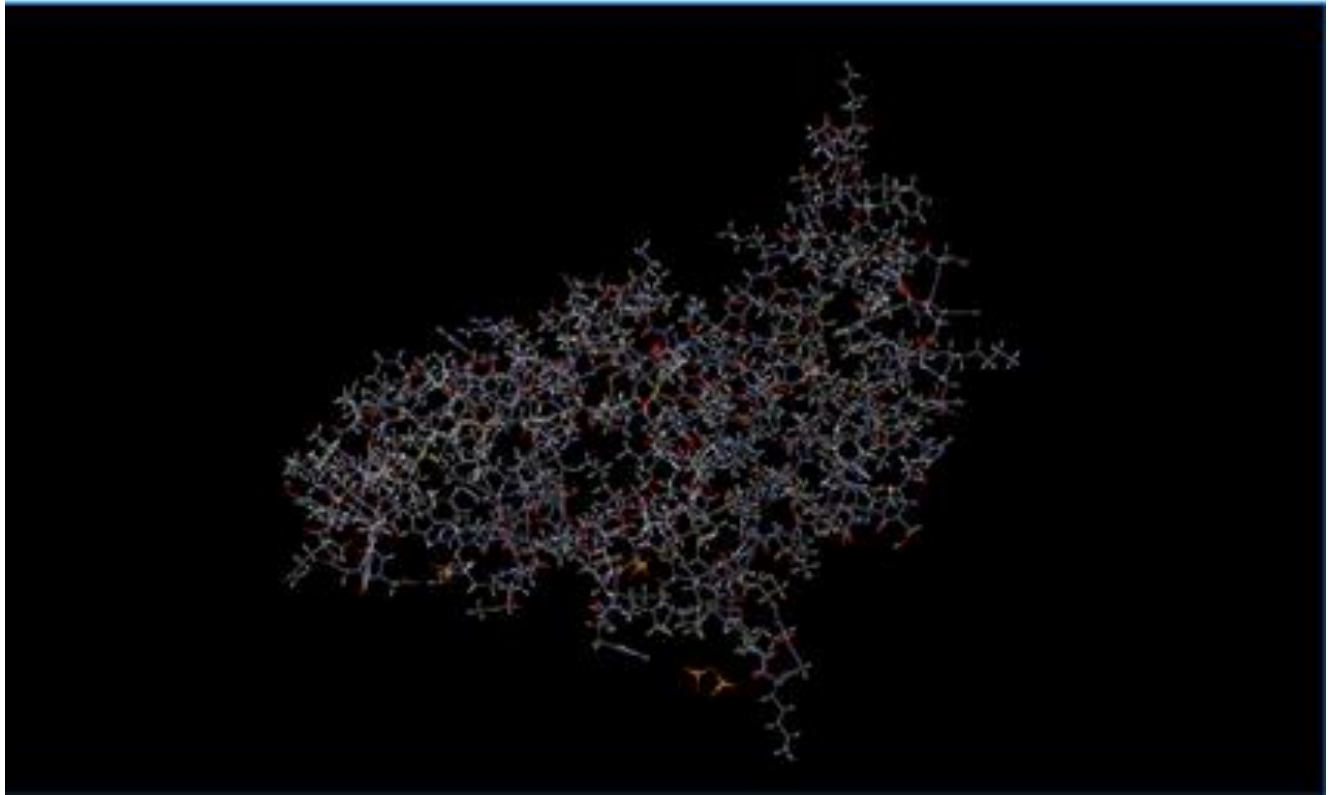


Fig (16): Structure of Activin

Activin is a dimer composed of two identical or very similar beta subunits. Inhibin is also a dimer wherein the first component is a beta subunit similar or identical to the beta subunit in activin. However, in contrast to activin, the second component of the inhibin dimer is a more distantly-related alpha subunit. Activin, inhibin and a number of other structurally related proteins such as anti-Müllerian hormone, bone morphogenetic protein, and growth differentiation factor belong to the TGF- β protein superfamily.

ACTIVIN AMINO ACID LABELS

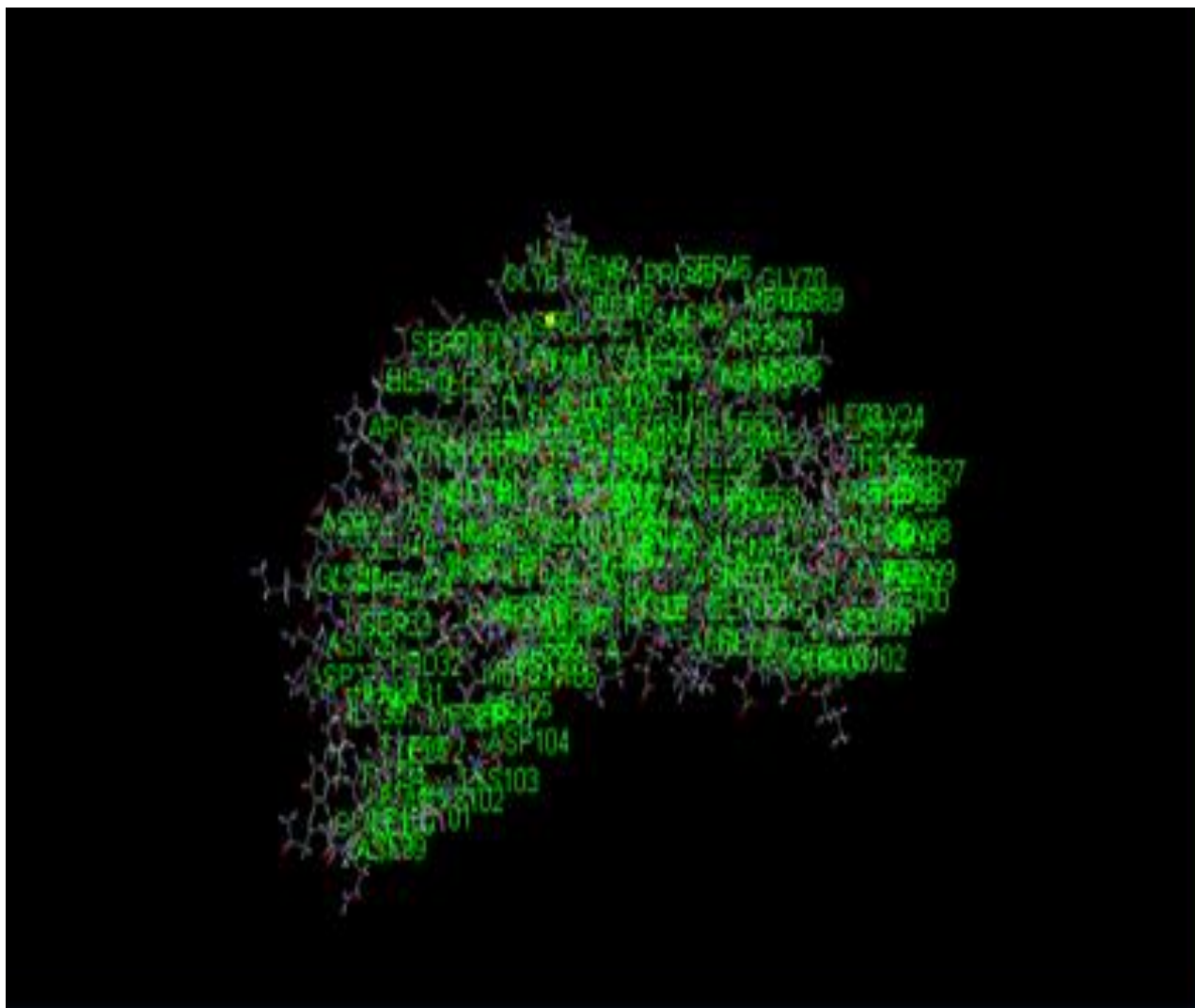


Fig (17): Amino acid labels of Activin

ACTIVIN BINDING SITES

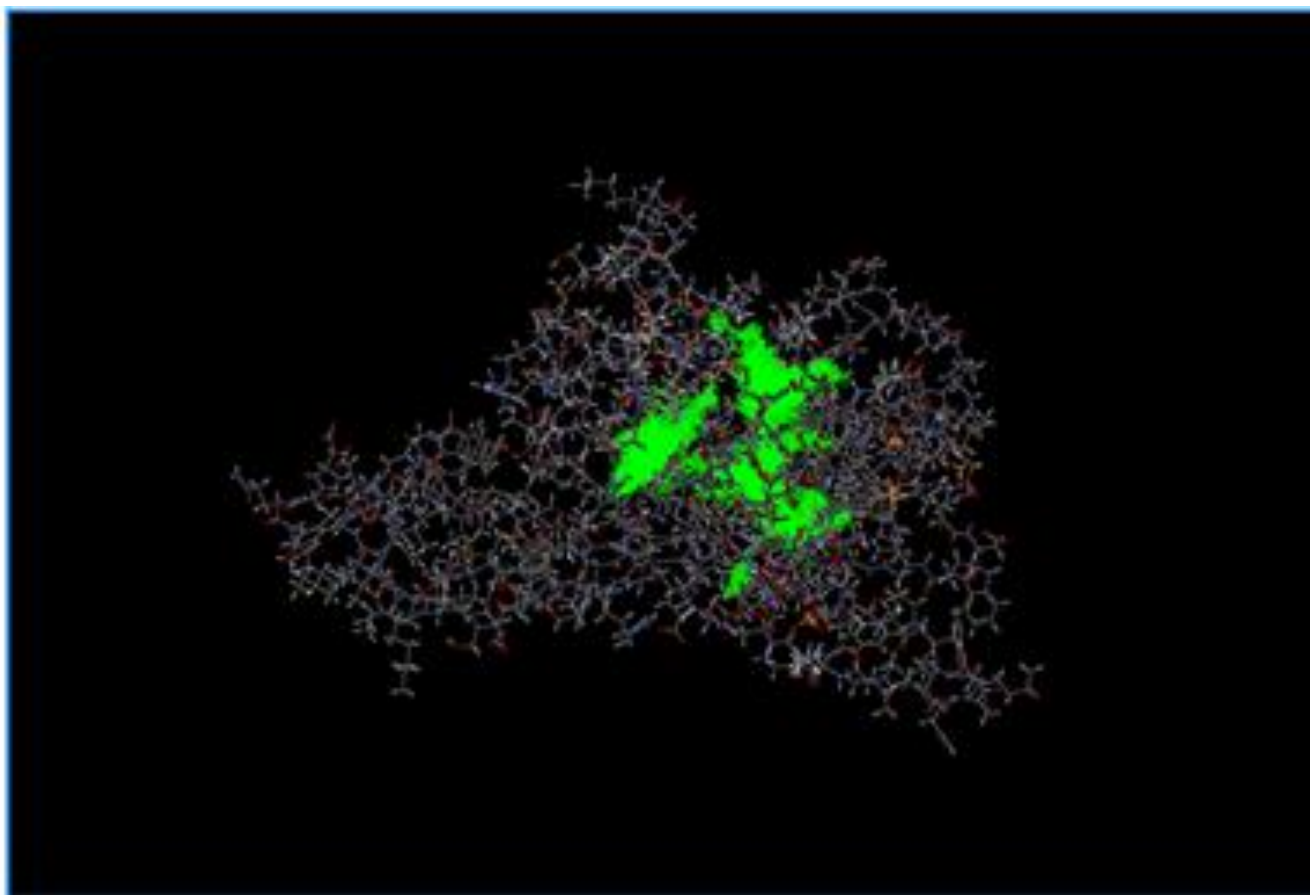


Fig (18): Activin binding sites

EPICATECHIN

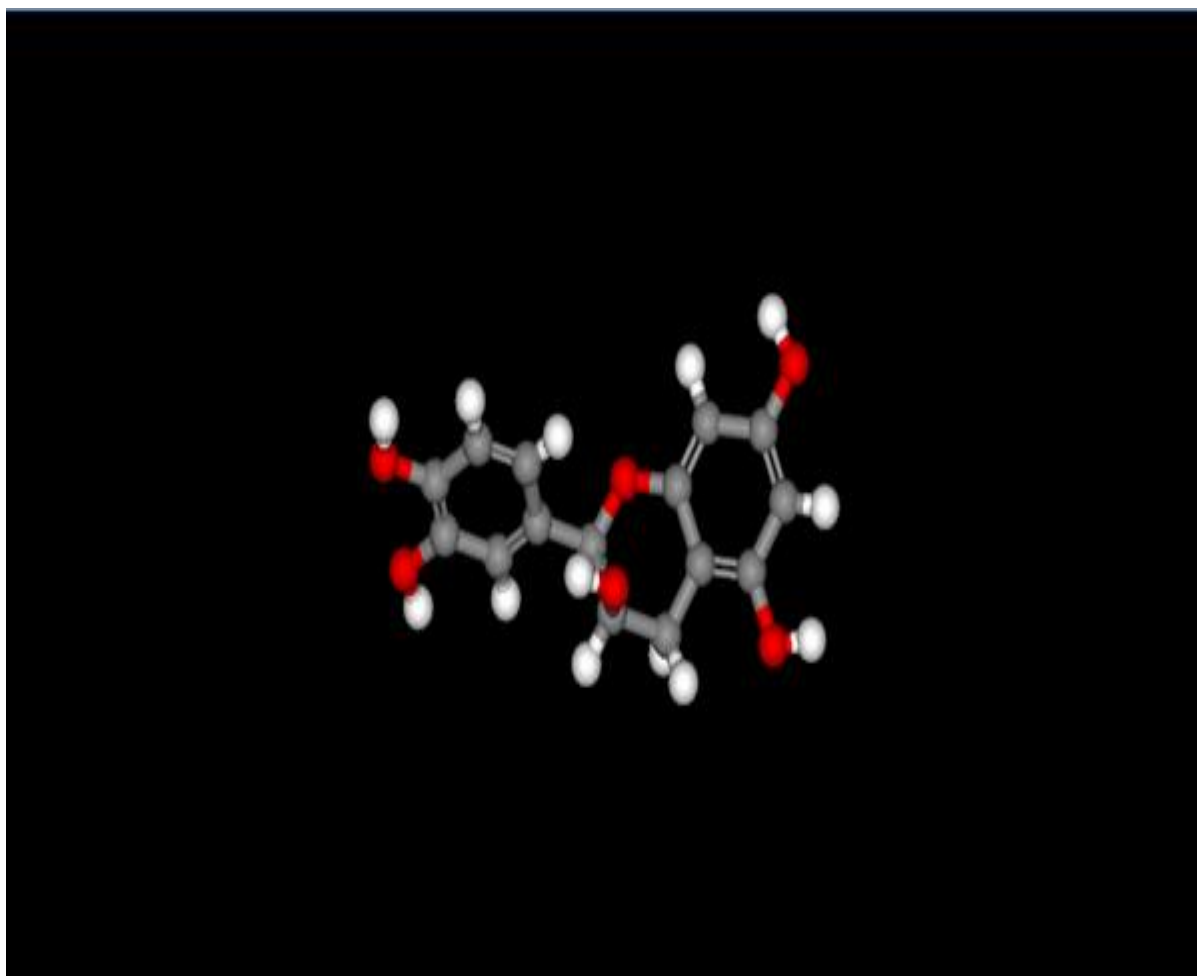


Fig (19): Structure of Epicatechin

ACTIVIN-EPICATECHIN DOCKING

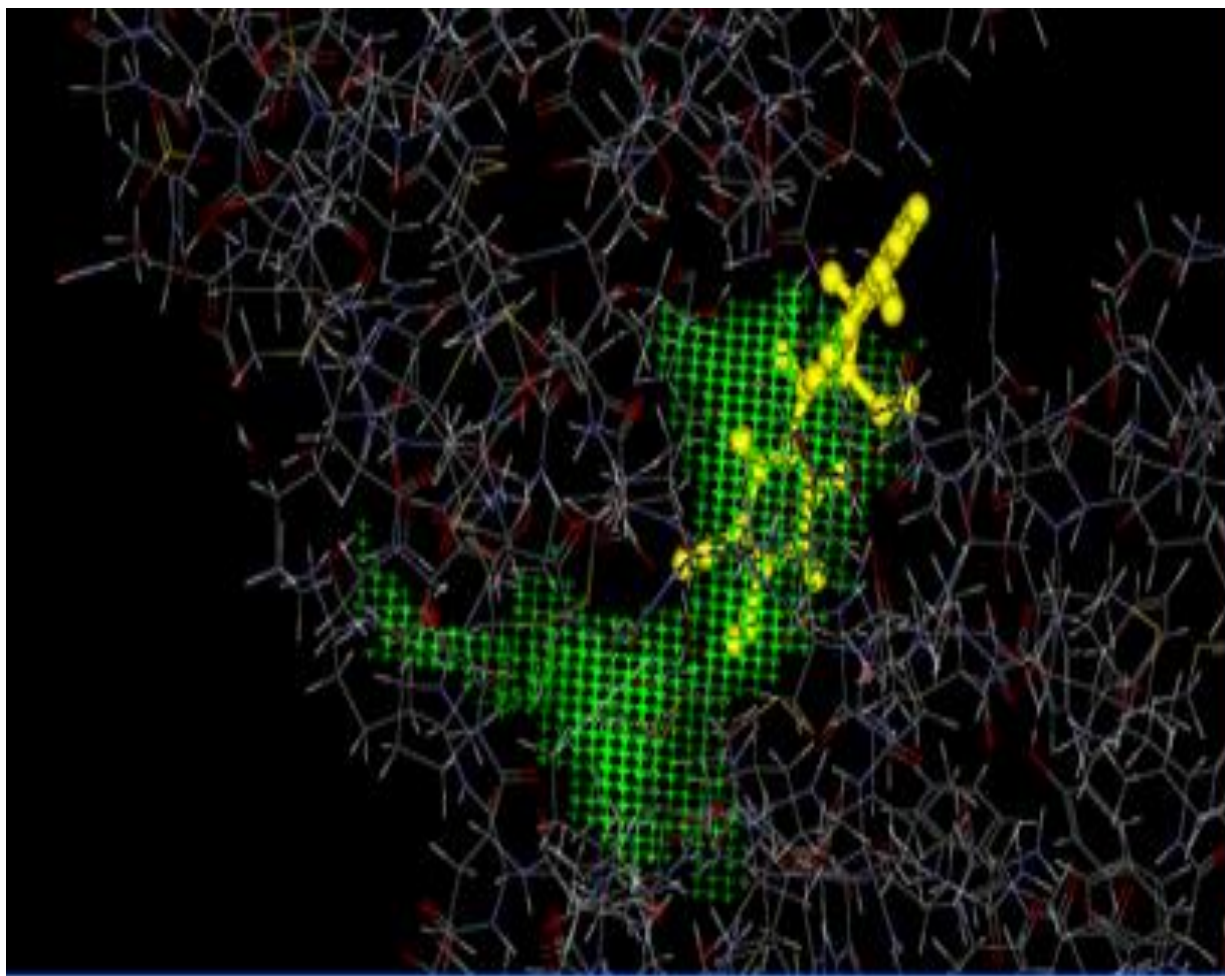


Fig (20): Interaction of Activin and Epicatechin

DOCK SCORE: **44.123**

LEUCOPELARGONIDIN

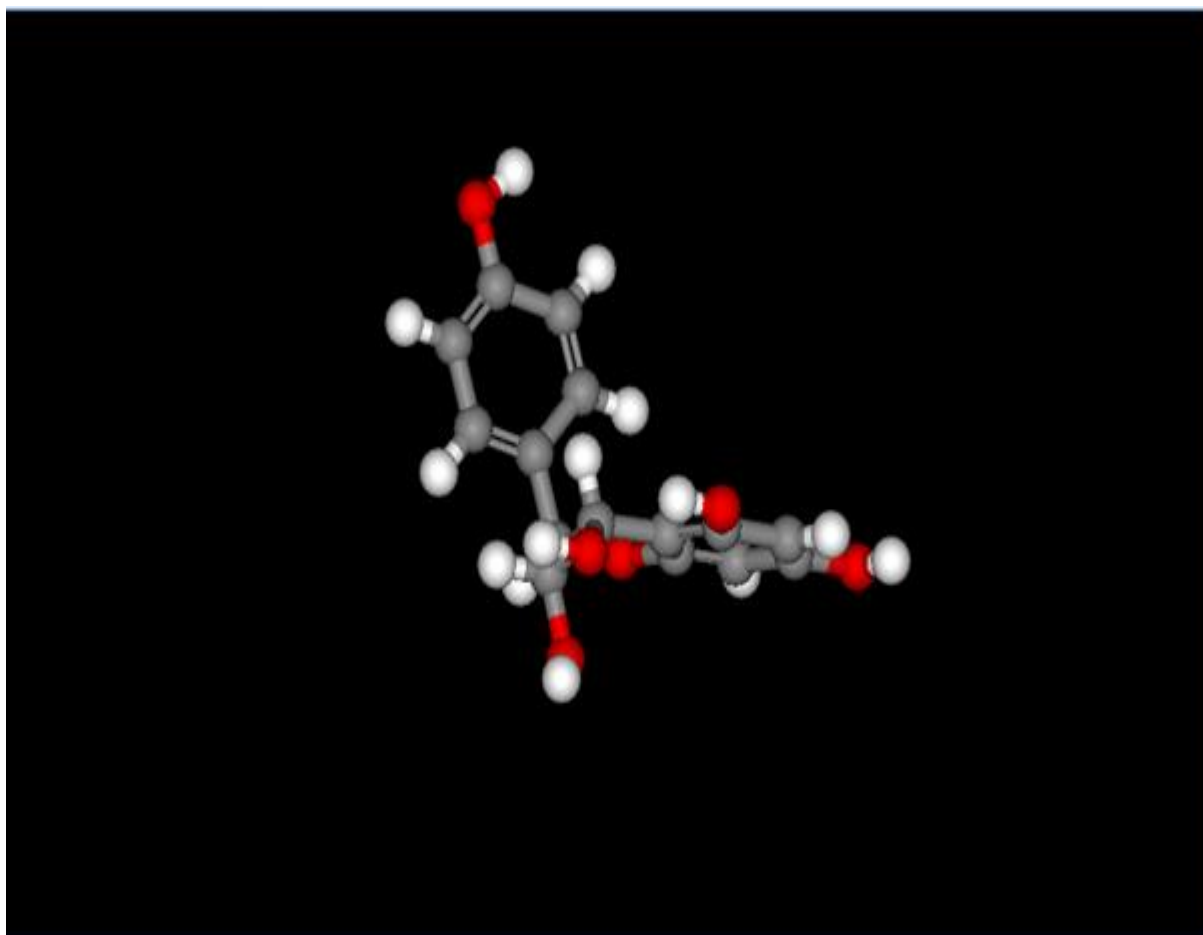


Fig (21): Structure of Leucopelargonidin

ACTIVIN-LEUCOPELARGONIDIN DOCKING

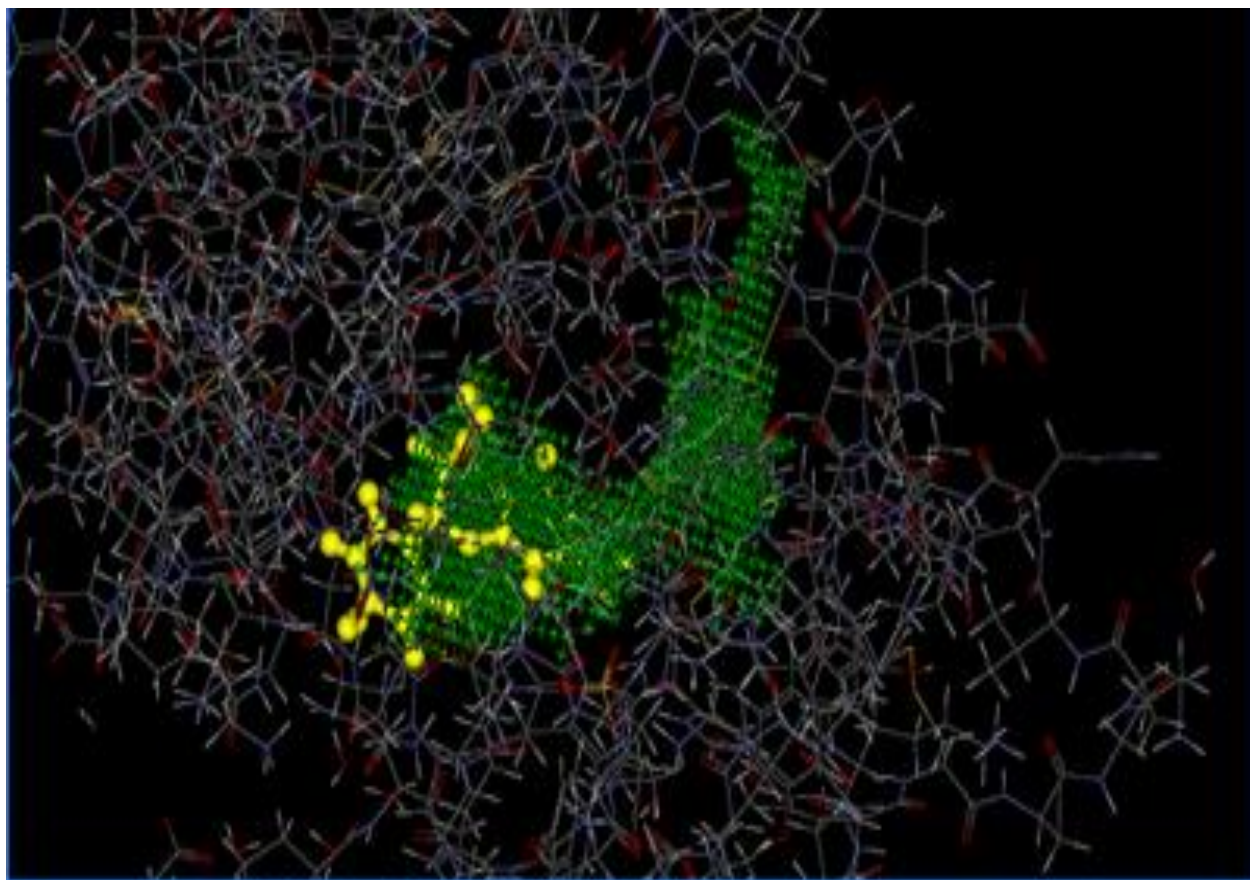


Fig (22): Interaction of Activin and Leucopelargonidin

DOCK SCORE: 12.078

LEUCOCYANIDIN

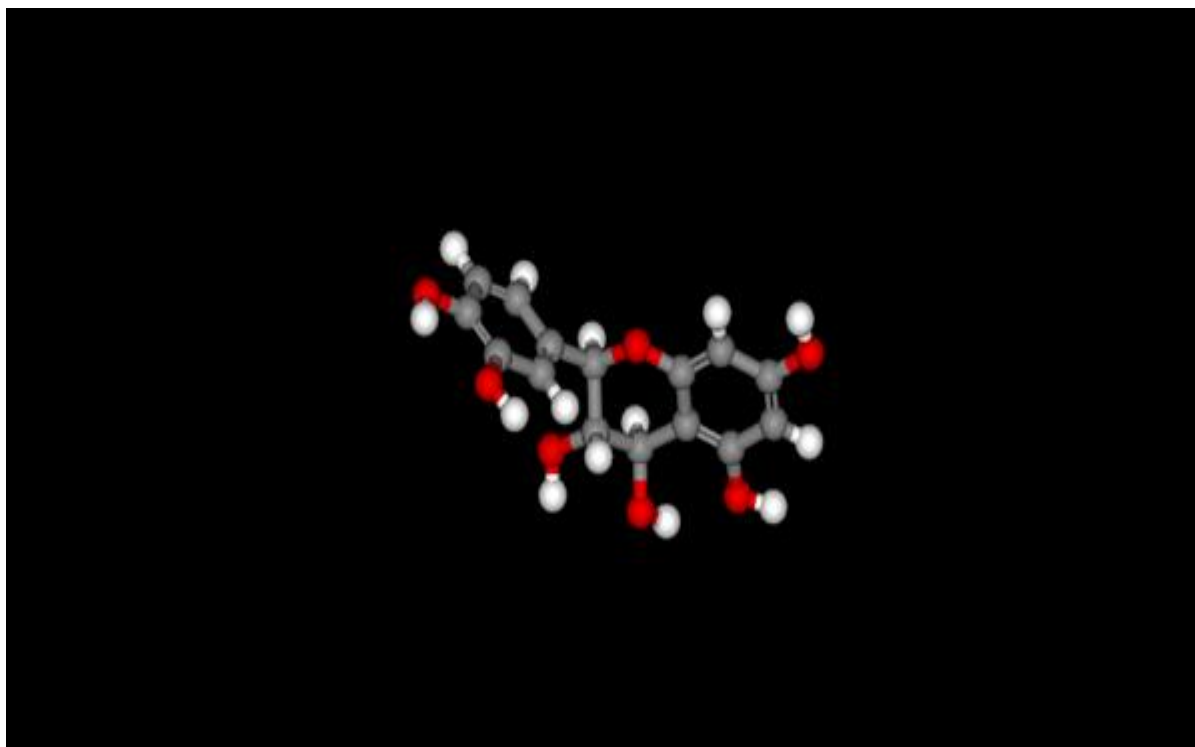


Fig (23): Structure of Leucocyanidin

ACTIVIN-LEUCOCYANIDIN DOCKING

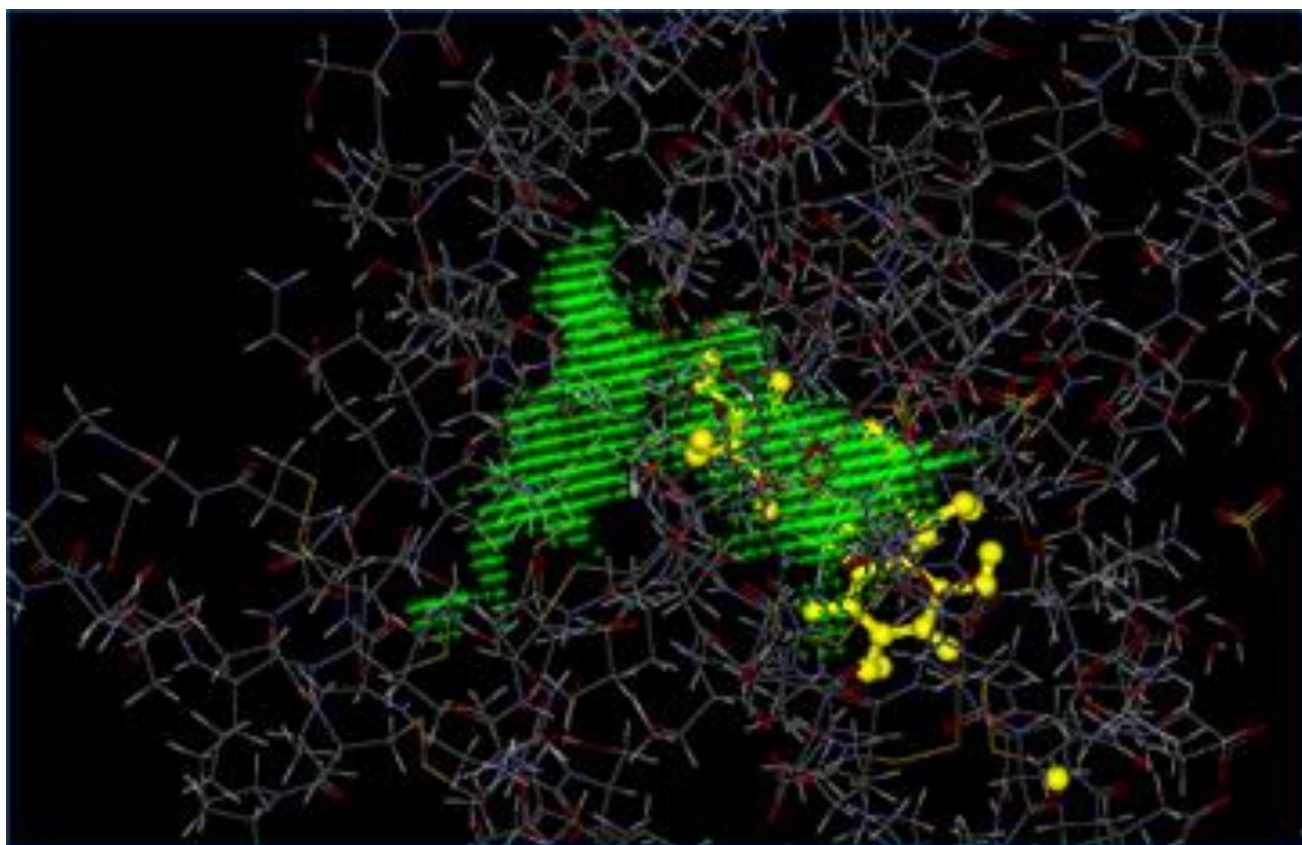


Fig (24): Interaction of Actin and Leucocyanidin

DOCK SCORE: 33.933

CATECHIN

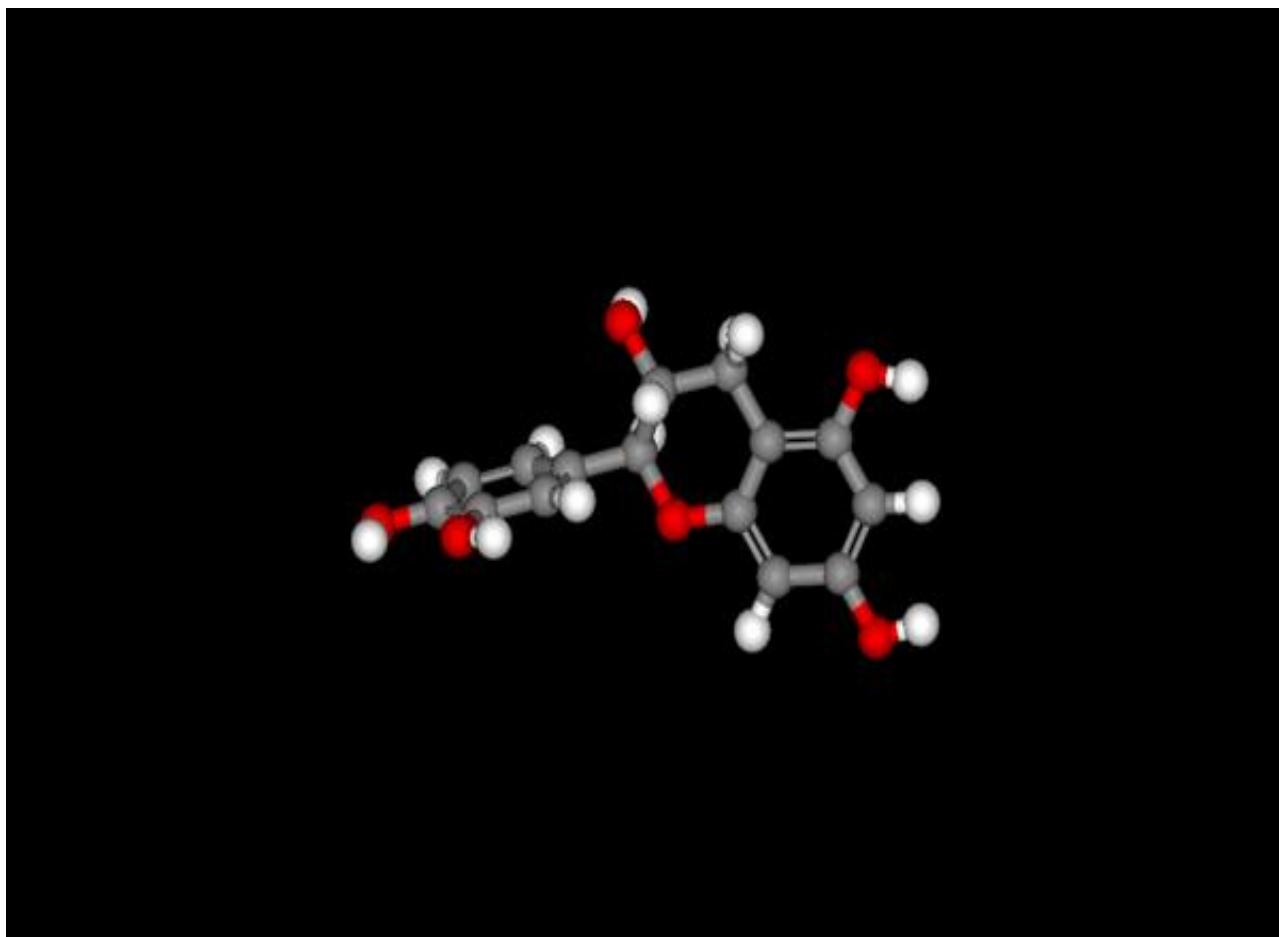


Fig (25): Structure of Catechin

ACTIVIN-CATECHIN DOCKING

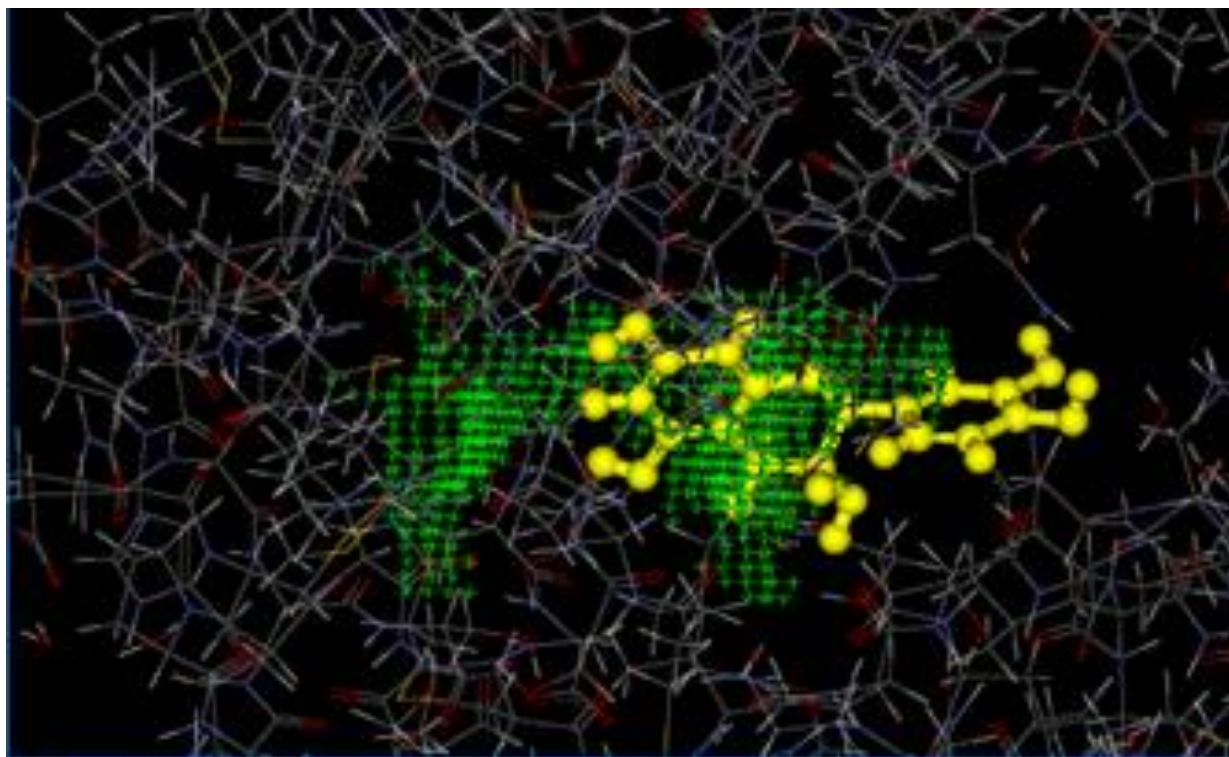


Fig (26): Interaction of Activin and Catechin

DOCK SCORE: 32.269

DOCKING SCORE RESULTS

TARGET	LIGAND	DOCK SCORE
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FOLLISTATIN	EPICATECHIN	40.723
	KAEMPFEROL	32.787
	LEUCOPELARGONIDIN	38.501
	LEUCOCYANIDIN	47.103
	CATECHIN	33.452
ACTIVIN	EPICATECHIN	44.123
	KAEMPFEROL	0
	LEUCOPELARGONIDIN	12.078
	LEUCOCYANIDIN	33.933
	CATECHIN	32.269

DISCUSSION

Docking was performed for proteins follistatin and activin with natural drugs using the software Accelrys Discovery Studio. These proteins were docked with 5 drugs against pcors derived from

the bark and flowers of ashoka tree. Based on energy minimization value among the 5 ligands which has the highest score is said to be the best docked molecule. The best dockind score was noted for these structures and table was created. Out of these drugs, leucyanidin gives the best docking score with follistatin and epicatechin gives the best docking score with activin for Polycystic ovarian syndrome.

Follistatin also known as activin-binding protein is a protein that in humans is encoded by the *FST* gene. Follistatin is an autocrine glycoprotein that is expressed in nearly all tissues of higher animals. It is a single chain glycosylated polypeptide that can bind to activin. When follistatin binds to activin it suppresses the role of activin to stimulate the secretion of Follicle Stimulating Hormone (FSH). FSH plays an important role in folliculogenesis and decrease in FSH level may arrest follicular development.

Activin is a dimer composed of two identical or very similar beta subunits. In the ovarian follicle, activin increases FSH binding and FSH-induced aromatization. It participates in androgen synthesis enhancing LH action in the ovary and testis. In the male, activin enhances spermatogenesis.

CONCLUSION

Natural products discovered from medicinal plants have played an important role in the treatment of Polycystic Ovarian Syndrome. Plant-derived compounds have played an important role in the

development of several useful drugs against Polycystic Ovarian Syndrome. These include epicatechin, kaempferol, leucopelargonidin, leucocyanidin and catechin. There are more than 270,000 higher plants existing on this planet and many diseases are treated with drugs that come from natural product sources. For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates and invertebrates. In this study, leucocyanidin and epicatechin have found to have significant role against Polycystic Ovarian Syndrome, because throughout the interaction studies both these drugs shown to have the best docking score for Polycystic Ovarian Syndrome. Hence leucocyanidin and epicatechin may be used as drug against Polycystic Ovarian Syndrome. Out of the 10 docking, the best docking scores are listed below

FOLLISTATIN-LEUCOCYANIDIN: 47.103

ACTIVIN-EPICATECHIN: 44.123

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