IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

In-Silico Driven Exploration of *Coccinia grandis*: A Dual Shield against Kidney Stones and Nephrotoxicity

Mr Prajyot Balu Ravat
Dr. Mahesh J. Patil
Student, Associate Professor
ASPM college of pharmacy sangulwadi

ABSTRACT

Medicinal plants are also found to be playing a central part in treating all kinds of health issues and remain a significant part of traditional medicinal practice. In this aspect, Coccinia grandis is an impressive species, traditionally being used by local people in the cure of many diseases. The plant is found to possess a very wide range of pharmacological activities such as antibacterial, analgesic, antispasmodic, anti-inflammatory, antipyretic, cathartic, and expectorant. Phytochemical compounds like alkaloids, glycosides, triterpenoids, phenols, flavonoids, saponins, and tannins are commonly present in its leaves.

The current work was conducted to isolate, purify, and identify active chemical constituents present in leaves of Coccinia grandis. The process of extraction was performed with the help of a Soxhlet apparatus and followed by preliminary phytochemical screening. Separation and purification were accomplished using Thin Layer Chromatography (TLC) and Column Chromatography. The isolated compounds were identified using UV and IR spectroscopy. The bioactive molecule was also studied for nephroprotective and antiurolithiatic activities against an in vitro model, as well as in silico studies using molecular docking methods to identify possible biological targets. The results indicate that Coccinia grandis has good potential for the development of herbal therapeutic agents against kidney disorders.

KEYWORDS: Coccinia grandis, Antiurolithiatic activity, Nephroprotective activity, Molecular Docking

1. INTRODUCTION

In recent years, there has been renewed global interest in traditional medicine, with many countries integrating its safe practices into national health systems. Historically, plants have been a major source of medicine, especially in India, where about 95% of traditional prescriptions are plant-based in systems like Ayurveda, Unani, Siddha, and Homeopathy. Coccinia, a climbing herb from the Cucurbitaceae family, native to India, Asia, and Central Africa, is known for its medicinal and nutritional value. Its seeds are rich in oil and protein, while its stem, tendrils, leaves, flowers, and red fruits are used for various purposes, including food and medicine.[4]

1.1 Relevance & Motivation:

The stem and root of the Coccinia species are best used for skin diseases, asthma, bronchitis, joint pain relief, and many other ailments. The most useful part of these plants is the leaves, which are classified as palmately simple with five lobes, although their shape varies from heart-shaped to pentagon-shaped. The leaves exhibit anti-diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, and cathartic and expectorant activities. Leaf extracts are also found to have hypoglycemic, hypolipidemic, and antioxidant effects. Coccinia species leaves play a significant role in the medicinal properties of plants belonging to the family Cucurbitaceae. The plant contains active constituents such as alkaloids, flavonoids, phenolic compounds, amino acids, lupeol, and proteins, which are responsible for its anti-lithic activity.6 In the present study, in-silico docking will be carried out for the compounds present in Coccinia species in comparison with the standard captopril against ACE protein.

Computer-aided drug design is used to determine whether the screened molecule binds to the target and if so how strongly it binds to the target molecule. Each year, more than half a million people go to emergency rooms for kidney stone problems. The prevalence of kidney stones in the United States increased from 3.8% in the late 1970s to 8.8% in the late 2000s. The prevalence of kidney stones was 10% during 2013–2014. The risk of kidney stones is about 11% in men and 9% in women. Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size (usually at least 3 millimeters), they can obstruct the ureter. Ureteral obstruction causes postrenal azotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces) and spasm of the ureter.[7] Thus the present study is focused in the direction of *in-silico* molecular docking, phytochemical investigation & possible pharmacological activities of *coccinia species.*

The Indigenous system of medicine namely Siddha Medicine, Ayurveda, Unanim, has existed for several centuries. The word siddha which means in sanskrit “realized”, one who is endowed with supernatural faculties called “siddhi”, refers to groups of tantric yogis who have acquired supernatural powers through austere ascetic practices. Siddha medicine is one of the traditional systems. Coccinia grandis, also called tindora' (tindori, tindoori) commonly known as “Kovai” belongs to the family Cucurbitaceae. Coccinia grandis, also called tindora' (tindori, tindoori) commonly known as “Kovai”. It is perennial climber with slender, cylindrical, glabrous stems and simple tendrils; There has been plenty of research work on this plant as evidenced by the literature review presented in this paper.[8]

1.2 MORPHOLOGICAL CHARACTERS

Synonyms: Coccinia cordifolia, Coccinia indica, Cephalandra indica, Physedra, Staphylosyce [9-10]

Table 1.1: Botanical Classification of Coccinia grandis.

Kingdom	Plantae
Order	Cucurbitales
Family	Cucurbitaceae
Sub family	Cucurbitoideae
Tribe	Benincaseae
Sub tribe	Benincasinae

Genus	Coccinia Wight & Arn.
Species	Coccinia indica

1.2.1 VERNACULAR NAMES

Table 2: Vernacular names of Coccinia grandis.[11-12]

Sanskrit	Tundika
Assam	Kawabhaturi
Bengal	Bimbu
English	Ivy-gourd
Hindi	Kundaru ki bel, Kundru
Punjab	Kanduri
Tamil	Kovai
Urdu	Kunduru
Gujrat	Ghilodi
Oriya	Parwal, Kundru, Tond
Malayalam	Tendli (Konkani), Ghiloda, Kundri, Kowai, Kovai, Kovakkai

1.2.2 DISTRIBUTION

Coccinia grandis (Ivy gourd) is occasionally cultivated as a garden vegetable in the tropical and sub-tropical regions of the world. It is believed to be native to central Africa, India, and Asia. Its long history of usage, cultivation, and transportation by people has obscured its base. It is a common weed in South-East Asia.It is considered a valuable wild vegetable by the indigenous people of Southeast Asia and India.[13-14]

1.2.3 BOTANICAL DESCRIPTION

Coccinia grandis is a perennial, glabrous, climbing herb or trailing vine with glabrous stems and tuberous roots. It is a fast-growing perennial vine that grows several meters long. It can form dense mats that readily cover the shrubs and small trees. Its leaves (Figure 1) are arranged alternately along the stems; Tendrils are simple. The plant is dioecious (male flowers are produced on separate plants to female flowers). Flowers (Figure 2) are large, white and star-shaped. The ovary is inferior. The fruit (Figure 3) becomes red (when ripe), ovoid to elliptical, 25–60 mm long, 15–35 mm in diameter, and hairless on stalks 10–40 mm long. Seeds are tan-colored and 6 –7 mm long. The roots and stems are succulent and possibly enables the plant to survive prolonged drought.[15]

Leaf	Flower	Fruit

Fig 1.1. Plant of Coccinia grandis

1.2.4 NUTRIENT COMPOSITION

Table 1.3: Nutrient composition of Coccinia grandis[16]

Carbohydrate	12.62%
Total protein	15%
Water soluble protein	11.25%
Lipid	4.00%
Total Phenol	61.92mg/100g
Vitamin C	25.55 mg/100g
β Carotene	70.05mg/100g
Potassium	3.3 mg/100g
Phosphorous	1.15 mg/100g
Sodium	0.95mg/100g
Iron	2.33 mg/100g
Calcium	3.79mg/100 g

1.2.5 CHEMICAL CONSTITUENTS

The plant contains resins, alkaloids, fatty acids, flavonoids and proteins as chief chemical constituents. Aspartic acid, Glutamic Acid, Asparagine, Tyrosine, Histidine, Phenylalanine, Threonine, Valine, and Arginine are also found. The methanolic extract of fruit contains alkaloids, steroids, tannins, saponins, ellagic acid, phenols, glycosides, lignans, and triterpenoids. Roots contain Triterpenoid, saponin coccinioside, Flavonoid glycoside ombuin 3-o- arabino furanoside, Lupeol, β-amyrin, and βsitosterol and Stigmast -7- en-3-one.[17]

It contains many chemical constituents in every of its part. They include:

I. **Aerial part:** - Heptacosane, Cephalandrol, β -β-sitosterol, Alkaloids Cephalandrins A and B.

II. **Fruits:** - β- Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, β-carotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3one.

III. **Root:-** Resin, Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, Saponin Coccinoside, Flavonoid Glycoside, Lupeol, β-amyrin, β- sitosterol, Taraxerol.[18-20]

1.2.6 MEDICINAL USES OF DIFFERENT PARTS

1. Leaf Antidiabetic, antioxidant, larvicidal, GI disturbances, cooling effect to the eye, gonorrhoea, hypolipidemic, skin diseases, urinary tract infection.

2. Fruit Hypoglycemic, analgesic, antipyretic, hepatoprotective, tuberculosis, eczema and anti-inflammatory.

1.3 PHARMACOLOGICAL ACTIVITIES

1.3.1 ANTI-BACTERIAL ACTIVITY

Umbreen Farrukh et al., investigated the In vitro antibacterial activity of leaves and stem extracts of Coccinia grandis against gram-positive (Bacillus cereus, Corynebacterium diphtheriae, Staphylococcus aureus and Streptococcus pyogene and gram-negative (Escherichia coli, The zone of inhibition of bacterial growth was measured and is compared with the control. Water extract of leaves and ethanol extract of the stem showed high activity against Shigella boydii and Pseudomonas aeruginosa equivalent to the reference drugs.[21]

1.3.2 HEPATOPROTECTIVE ACTIVITY

Vadivu et al., evaluated the hepatoprotective activity of alcoholic extract of the fruits of Coccinia grandis using carbon tetrachloride (CCl4)- induced hepatotoxicity in rats. Male Wistar strain albino rats weighing 150 – 200 g and albino mice weighing 22 –25 g were used. 24 rats were taken for the study, divided into 4 groups of 6 animals. The levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein, total and direct bilirubin were evaluated in experimental rats (with or without CCl4-induced hepatotoxicity) following administration of an alcoholic extract of the fruits of C. grandis using standard procedures. The potency of the extract was compared with the standard drug silymarin at a dose of 100 mg/kg p.o. Histopathology of the liver tissues treated with the extract was also studied. At a dose level of 250 mg/kg, the alcoholic extract significantly decreased the activities of serum enzymes (SGOT, SGPT, and ALP) and bilirubin which were comparable with that of silymarin.[22]

Anusha Bhaskar et al., studied on the Protective effect of Coccinia grandis fruit extract against (Diethylnitrosamine) DEN-induced Hepatotoxicity in Wistar Albino Rats. They were divided into 5 groups consisting of 6 rats in each group. An elevated level of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP)and Alcohol dehydrogenases (ADH) were observed. Liver oxidative stress was confirmed by the elevation of lipid peroxidation that was measured as malondialdehyde (MDA), and a decrease in the enzymic and non-enzymic antioxidant activities. Oral administration of the methanolic fruit extract of Coccinia grandis for 30 days to DEN-treated rats significantly improved the antioxidant levels, reduced the oxidative stress and also caused a reversal of the liver parameters. The results obtained were comparable with that of the standard drug silymarin.[23]

1.3.3 ANTI-ULCER ACTIVITY

Thirupathi et al., carried out a study on the Antiulcer activity of ethanolic, aqueous and total aqueous extracts of coccinia grandis leaf in pyloric ligature-induced ulcers in albino rats. The ulcer was induced by

pylorus ligature. Rats were divided into 8 groups with six each. Drugs were administered in two different dose levels (200mg/Kgbwt, and 400mg/ Kgbwt). .[24]

1.3.4 ANTI-OXIDANT ACTIVITY

Tapan Kumar Chatterjee et al., investigated on the effect of the fractions of Coccinia grandis leaf extract on lipid peroxidation and antioxidant enzymes in oxonateinduced Hyperuricaemic mice. Swiss albino mice of either sex were used. They were divided into 7 groups consisting of six each. The petroleum ether, chloroform, ethyl acetate, and residual fractions of the hydromethanolic extract of the leaves of coccinia grandis at a dose of 200 mg/kg bw were given orally to the mice for 7 days. Potassium oxonate, was injected intraperitoneally (280 mg/ kg) to induce hyperuricemia. The end products of lipid peroxidation, Malondialdehyde (MDA) and lipid hydroperoxides (LH) and the levels of tissue protein, enzymatic and nonenzymatic antioxidants were estimated in the liver. Allopurinol (10mg/kg) was used as the standard. Significant elevation was noted in MDA and LH and a decrease in total protein and antioxidant enzymes in hyperuricaemic mice compared to normal control. Among the fractions tested, the chloroform fraction demonstrated the highest activity followed by the petroleum ether and ethyl acetate fractions.[25].[26]

1.3.5 ANTI-DIABETIC ACTIVITY

Saikat Ghosh et al., evaluated the antidiabetic potential of methanolic extract of Coccinia indica leaves in streptozotocin (65 mg/ kg) i.p induced adult Wistar strain albino diabetic rats. Normoglycemic and Antihyperglycemic studies were conducted. 18 rats divided into three groups of six animals and each was subjected for Normoglycemic study. Group II and III, were given methanolic extract of C. indica (MECI) at concentrations of 150 and 300 mg/kg, by oral route. The glucose levels were found to be rising in the rats of the control group over a period of 12 hrs from treatment by 5.33% when compared to the results after 1 hr of treatment. In case of the MECI-treated rats of Group II and III over the same period of time and upon a similar comparison, the glucose levels were found to have decreased by 27.58 and 26.24% respectively, whereas in Antihyperglycaemic Study: .[27]

1.3.6 ANTI-TUSSIVE ACTIVITY

Shakti Prasad Pattanayak et al., studied the In vivo antitussive activity of Coccinia grandis fruit against irritant aerosol and sulfur dioxide-induced cough model in rodents. The experiments were carried out in male guinea pigs (400-450 g) and Swiss albino mice(30-40 g) of either sex. Guinea pigs, five in each group, and mice divided into five groups, each containing 10 mice were used for the study. 10 min after exposing the male guinea pigs to aerosols of the test solutions for about 7 minutes. Also,the methanolic extract exhibited significant antitussive effect at 100, 200 and 400 mg/kg, per orally by inhibiting the cough by 20.57, 33.73 and 56.71% within 90 min of performing the experiment.[28]

1.3.7 ANTIHYPERLIPIDEMIC ACTIVITY

Dewan Md. Sumsuzzman et al., studied the Antihyperlipidemic activity of coccinia grandis leaf extracts on high-fat diet induced Wistar albino rats. Male Wistar albino rats were divided into four groups: Groups I normal control; Group II HFD control; Group III HFD + C. grandis extract (2 mg/gm), Group IV HFD + Olive oil (2 mg/gm). The whole study lasted for about 5 weeks. Administration of HFD caused a significant increase in the serum total cholesterol (T.C), LDL-cholesterol, VLDLcholesterol, triglycerides (T.G). [30]

1.3.8 ANTI-INFLAMMATORY ACTIVITY

Deshpande et al., carried out a study on the Antiinflammatory activity of the leaf and stem aqueous extracts of coccinia grandis. Sprague Dawley rats (120- 150 g) and Swiss albino mice (40-50 g) were used for the study. After one hour the last dose was administered; 0.2 ml of formaldehyde (1%, w/v) injected into the rat hind paw. Aqueous extract of the leaves showed a more significant percentage inhibition of paw edema than the aqueous extract of stem and standard, used as indomethacin. So Coccinia grandis can be thought to possess antiproliferative and antiarthritic activities similar to indomethacin.[31] .[32]

1.3.9 ANALGESIC AND ANTIPYRETIC ACTIVITY

Aggarwal Ashish S et al. investigated the Analgesic and antipyretic activity of methanolic extract (50, 100 and 200 mg/kg) of Coccinia grandis leaves in experimental rats and mice. Wistar rats (200-250 g) of either sex or Albino mice (Swiss strain) weighing 22-25 g were used for the study. Acetic acid induced writhing, Tail immersion, and Hotplate models were used to evaluate analgesic activity and Yeast induced pyrexia model was used to evaluate antipyretic activity. Findings showed that oral administration of methanol extracts significantly inhibit acetic acid-induced writhing in mice in a dose-dependent manner but failed to show significant inhibition in Tail immersion and Hotplate models. The antipyretic study revealed that the methanolic extract exhibits a significant reduction in pyrexia that was comparable to the standard drug.[33]

1.3.10 ANTICANCER ACTIVITY

Bolay Bhattacharya et al., carried out the In vivo and in vitro anticancer activity of Coccinia grandis on Swiss albino mice. Anticancer activity of the plant extract against Ehrlich Ascites Carcinoma (EAC) cell on mice was carried out. The mice were divided into four groups each group consisting of 10. Ethanol extracts and vinblastine (reference drug) were injected intraperitoneally. After treatment for nine days, mice were weighed and sacrificed on the 10th day. Viable (live) and nonviable (dead) cell counting, intraperitoneal fluid volume, packed cell volume, hemoglobin concentration, .[34]

1.3.11 LARVICIDAL ACTIVITY

SI Mohammed et al., carried out the Evaluation of Larvicidal Activity of Essential Oil from the leaves of Coccinia grandis against three Mosquito Species. The essential oil was extracted by hydro distillation using Clevenger apparatus and was analyzed for chemical constituents by gas chromatography-mass spectrometry (GC-MS). Larvicidal activity was recorded after 12th and 24th hr of post-exposure against the three mosquito species, Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Dead larvae were identified as they failed to move after probing with a needle in the siphon or cervical region. The LC50 and LC90 values of the three mosquito larvae were calculated by Probit analysis. GC-MS analysis revealed that the essential oil contains 23 different constituents. Out of the 23 constituents, major constituents identified were ntetracosane (39.18%), n-eicosane (30.04%), tetratriacotane (2.97%), 7-oc-tadecanal (2.81%), and tricosane (2.31%). Essential oil from leaves of Coccinia grandis exhibited considerable larvicidal activity against An. stephensi with LC50 and LC90 values 39.41ppm and 123.24ppm. This was followed by Ae.aegypti and Cx. quinquefasciatus with LC50 and LC90 values of 48.20ppm, 131.84ppm and 52.80ppm, 135.48ppm, after 24hrs of exposure. [35]

1.4 MAST CELL STABILIZING, ANTIANAPHYLACTIC, AND ANTIHISTAMINIC ACTIVITY

passive cutaneous anaphylaxis in rats and for clonidine-induced catalepsy in mice. ECGF at (100−150 mg·kg−1, i.p.) significantly protected egg albumin induced degranulation of mast cells and caused reduction of blue dye leakage in passive cutaneous anaphylaxis in a dose dependent manner. The treatment ECGF also inhibited the clonidine-induced catalepsy in a dose-dependent manner. Phytochemical studies observed the presence of saponin, steroids, alkaloids, flavonoids, and glycosides.[36]

1.4.1 MACROSCOPIC/MORPHOLOGICAL CHARACTERS

Its perennial herbs with tuberous root stock produce annual stems up to several meters long, which is found spreading on ground and twining around the tress and supports around it. Leaves are triangular or pentagonal in shape. Margin is dentate, upper surface glabrous and attachment of petiole and major vein branching occurs. Apex obtuse, petioles (1-3 cm) long and tendrils are unbranched. Flowers are monocots, solitary, rarely in axillary clusters of 2-3, pedicels (10-15 mm) long. Fruits are slimy in touch, pulpy and ovoid to ellipsoid shape. It is green in color when young and it turns scarlet red when it ripens (2.5-5 cm) long and (1.3-2.5 cm) in diameter, glabrous, purple red. .[37-38]

1.4.2 UROLITHIASIS

Urolithiasis is also called Nephrolithiasis or kidney stones. Urolithiasis is the formation of calculi, or condition associated with urinary calculi. The term calculi are synonymous with uroliths, stones, or crystals. These calculi are formed by deposits of polycrystalline aggregates composed of varied amount of crystalloid and organic matrix. Common components of calculi include Calcium oxalate (pure), Calcium oxalate in combination with as part ate, uric acid (pure), Calcium oxalate in combination with uric acid, Calcium oxalate dihydrate in combination calcium phosphate. In this present study three plants are selected for the anti urolithiasis study i.e. Aerva javanica, Punica granatum, Psidium guajava are made alcohol extracts. In vitro anti oxidant activity and Anti urolithiasis activity was performed by Hydrogen peroxide radical scavenging (H2O2) assay, Calcium Phosphate assay for Anti oxidant and Anti Urolithiasis activity respectively.[39] Urolithiasis is a global problem affecting human beings for several centuries. It is also called Nephrolithiasis or kidney stones. Urolithiasis isone of the major diseases of the urinary tract and is a major source of morbidity. Stone formation is one of the painful urologic disorders that occur in approximately 12% of the global population and its re-occurrence rate in males is70-81% and 47-60% in female.[40]

1.4.3 PATHOPHYSIOLOGY OF URINARY STONE

The stone formation requires supersaturatedurine, The Kidney stones contain calcium,oxalate, phosphate, magnesium, uric acid andthe formation of urinary stones involves acrystallization process it includes Nucleation,Growth and Aggregation of crystals.

Types of kidney stones

The Kidney stones are categorized on the basisof their type which includesa)

a) **Calcium stones4**: The most common type of calcium stones are calcium hydrogen phosphatedihydrate (Brushite crystals) and calciumoxalate. In most people, the kidneys excrete theextra calcium with the rest of the urine. Forsome people the calcium gets accumulated andaggregates with other products to form a stone.

b) Struvite stones: These stones normallydevelop after an infection in the urinary system.These stones contain the mineral magnesium and the waste product ammonia.

1.4.5 The Kidney

Structure of the kidney

The adult human kidney weighs about 160 g and measures approximately 11.25 cm in length, 2.5 cm in thickness, and 5.5–7.7 cm in width. These paired, bean-shaped, reddish-brown retroperitoneal organs are located between the twelfth thoracic and third lumbar vertebrae on either side of the vertebral column. They are protected by three tissue layers: (1) the innermost renal capsule, a tough fibrous layer guarding against shock and infection, (2) the adipose capsule, a fatty layer stabilizing kidney position, and (3) the renal fascia, outer fibrous tissue anchoring the kidney to the abdominal wall. A bisected kidney reveals two distinct regions: the pale renal cortex (outer) and the darker renal medulla (inner).41-42

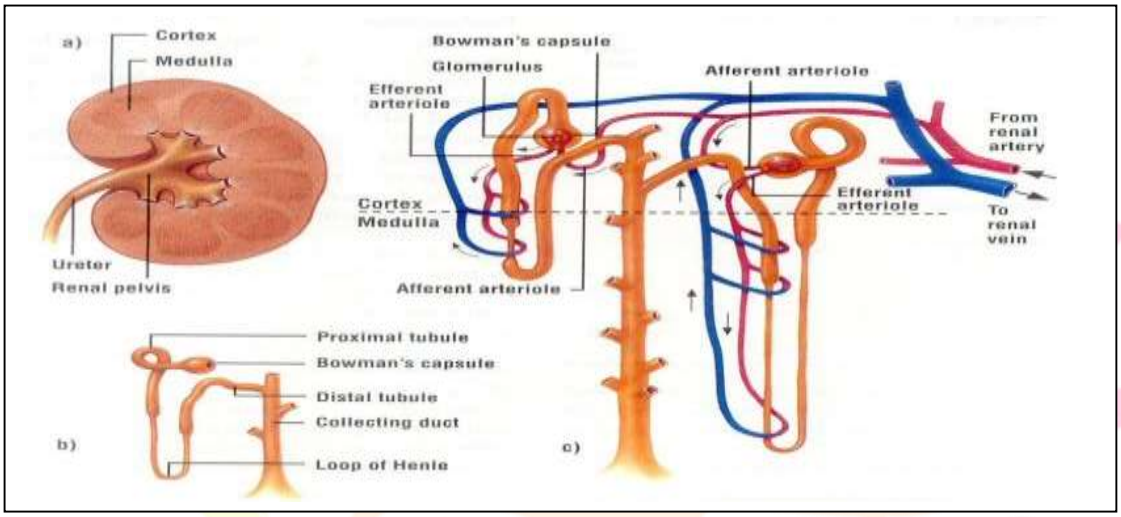


Figure 1.4: Structure of kidney & nephron

AIMS AND OBJECTIVES

Aim

In-Silico Driven Exploration of *Coccinia grandis*: A Dual Shield against Kidney Stones and Nephrotoxicity

Objective

1. To collect selected plant from Sangulwadi, Dist- Sindhudurg.
2. Collection of plant *Coccinia* grandis authentication.
3. Drying and milling of the plant material, methanol extraction of selected plant material.
4. To study silent features (morphological)
5. To extract the selected plants to determine antiurolithiatic and nephroprotective of the selected plant.
6. To study of Molecular Docking by *in silico*.

5. PLANT PROFILE

`

Figure 5.1: *Coccinia* Grandis.

Coccinia grandis, ivy gourd, also known as baby watermelon, little gourd, gentleman's toes, tindora. It is also known as Cephalandra indica and Coccinia indica.

Table 5.1: Taxonomical Status

Classification	**Coccinia grandis**
Kingdom	Plantae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Coccinia
Species	C. grandis
Binomial name	Coccinia grandis L(Viogt)
Common Names	Kanduri, Bimbi Kundru, Bimbaka , Kovai, Kundaru

1.5.1 Vernacular names

Hindi - Kunduru
Malayalam - Kova
Marathi - Tondli
Telugu - Tondikay
Tamil - Kovai
Sanskrit – Bimbika

1.5.2 Plant Description

Leaves are 5-10 cm, long and broad, bright green above, paler beneath, studded and sometimes rough with papillae, palmately 5-nerved from a cordate base, often with circular glands between the nerves, obtusely 5-angled or sometimes deeply 5-lobed, the lobes broad, obtuse or acute, apiculate, more or less sinuate toothed, petioles 2-3.2 cm long. Male flowers: Peduncles are 2-3.8cm.long and subfiliform. Calyx-tube is

glabrous, broadly campanulate and 4- 5 mm. long. Corolla is 2.5 cm. long, veined, pubescent inside and glabrous outside. Female flowers: Peduncles are 1.3-2.5cm long. Ovary is fusiform, glabrous and slightly ribbed. Fruits are fusiform-ellipsoid, slightly beaked, 2.5-5 by 1.3-2.5 cm. sized, marked when immature with white streaks, bright scarlet when fully ripe. Seeds are obovoid and rounded at the apex, slightly papillose, much compressed and yellowish grey.

1.5.3 Ethnomedicinal uses [53-55]

Coccinia grandis is a wild cucurbitaceous medicinal plant with many pharmaceutical applications. As an ethnic tribal plant, it has potential therapeutic values as anti diabetic, anti-ulcer, anti-inflammatory, anti-oxidant and anti-tumor properties. grandis such as roots, leaves and fruits are used for numerous medicinal purposes like wound healing, ulcers, jaundice, diabetes and antipyretic. [56-58]

1.5.4 Geographic spread

Its native range extends from Africa to Asia, including India, the Philippines, Cambodia, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam, eastern Papua New Guinea, and the Northern Territories, Australia. Its documented introduced range includes the Federated States of Micronesia, Fiji, Guam, Saipan, Hawaii, the Marshall Islands, Samoa, Tonga, and Vanuatu.[59]

Long-distance dispersal is most commonly carried out by humans due to its culinary uses or by mistake. Regarded as very invasive and on the Hawaii State Noxious Weed List, ivy gourd can grow up to four inches per day. It grows in dense blankets, shading other plants from sunlight and highjacking nutrients, effectively killing vegetation underneath.[60] It was introduced to Hawaii as a backyard food crop. It is sometimes tolerated along garden fences and other outdoor features because of its attractive white flowers. It has escaped to become a vigorous pest in Hawaii, Florida, Australia, and Texas.

1.5.5 Botany

Coccinia grandis is a fast-growing perennial vine that grows several meters long. It can form dense mats on lands that readily cover shrubs and small trees.

1.5.5.1 Leaves

Its leaves are arranged alternately along the stems; the shape of the leaves varies from heart to pentagon shaped. (Up to 10 cm wide and long). The upper surface of the leaf is hairless, whereas the lower is hairy. There are 3–8 glands on the blade near the leaf stalk. Tendrils are simple. Coccinia grandis is dioecious.

1.5.5.2 Flower

Flowers are large, white and star-shaped. The calyx has five subulate, recurved lobes, each 2–5 mm long on the hypanthium; peduncle 1–5 cm long. The corolla is white, campanulate, 3–4.5 cm long, deeply divided into five ovate lobes. Each flower has three stamens. The ovary of the Coccinia grandis flower is inferior.

1.5.5.3 Fruit

The fruit is red, ovoid to elliptical, 25–60 mm long, 15– 35 mm in diameter, glabrous, hairless on stalks.

1.5.5.4 Seeds

6-7 mm long, tan-colored, margins thickened.

1.5.5.5 Root

The roots and stems are succulent, tuberous and most likely facilitate the plant to survive prolonged drought. Desperations of Coccinia grandis are by the humans. Also spread by birds and other animals, pigs, moved unintentionally on equipment or on wood and germinate where they land. Hybridization and clonal selection are one of the viable methods to develop improved Clone in ivy gour.[61-62]

1.5.6 Chemical Constituents

Aerial part - Heptacosane, Cephalandrol, β -sitosterol, Alkaloids Cephalandrins A and B, Fruits- β- Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, β-carotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3-one. Root - Resin, Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, Saponin Coccinoside, Flavonoid Glycoside, Lupeol, β-amyrin, β-sitosterol, Taraxerol.[63]

1.5.7 Medicinal value of various parts of coccinia grandis

Table 2: Medicinal value of various parts of Coccinia grandis

Plant part	Medicinal value
Leaf	Antidiabetic, oxidant, larvicadal, GI disturbances, Cooling effect to the eye, Gonorrhea, hypolipidemic, skin diseases, urinary tract infection.
Fruit	Hypoglycemic, analgesic, antipyretic, hepatoprotective, tuberculosis, eczema. anti-inflammatory.
Stem	Expectorant, antispasmodic, asthma, bronchitis, GIT disturbances, urinary tract infection, skin diseases.
Root	Hypoglycemic, antidiabetic, skin diseases, removes pain in joint, urinary tract infection.

1.5.8 Pharmacological Activities

1.5.8.1 Antibacterial

Bhattacharya et al., (2010) evaluated the aqueous extract of leaves of Coccinia grandis for antibacterial activity against Shigella flexneri NICED, Bacillus subtilis Escherichia coli, Salmonella choleraesuis, Shigella dysenteries, and Shigella flexneri.[64] Aqueous extract of Coccinia grandis showed more significant antibacterial activity in comparison to ethanol extract. A polar moiety of the extract is more responsible for antibacterial properties. The chloroform extracts of Coccinia cordifolia moderately active against Sarcina lutea, Bacillus subtilis. Ethyl acetate extracts active against staphylococcus aurous. Hexane extract active against the sarcina lutea, Pseudomonas aeruginosa.[65]

1.5.8.2 Anthelmintic

Methanolic extract of Coccinia grandis posses the anthelmintic activity. The worm pheretime posthuma were used for Antihelmintic activity. Different concentrations of the extract are used. Methanolic extract of Coccinia grandis acts through paralyzing the worm. The activity is measured by the time taken to paralyzing the worm and death.[66]

1.5.8.3 Antioxidant

Moideen (2011) evaluated Ethanol extract of root of Coccinia grandis contain flavonoids which are responsible for antioxidant activity. Methanol extracts of the fruit of Coccinia grandis posses the potent antioxidant activity. The methanol extract of Coccinia grandis contains glycoside and flavonoid. The antioxidant activity of Coccinia grandis is due to the reducing power ability, hydrogen peroxide scavenging potential.[67]

1.5.8.4 Antiulcer The anti-ulcer activity

Aqueous extract of leaves of Coccinia grandis was investigated in pylorus ligation and ethanol induced ulcer models in experimental rats. Ulcer index was determined in both models. Aqueous extract of Coccinia grandis at doses of 250 and 500 mg/kg produced significant inhibition of the gastric lesions induced by pylorus ligation-induced ulcer and ethanol induced gastric ulcer .The extract showed significant reduction in ulcer index, free acidity and gastric.[68]

2. MATERIAL AND METHODS

A. Plant Material:- The *Coccinia* grandis were used for experimental purpose. They are collected from Sangulwadi, Dist Sindhudurg, India.

B. Chemicals:-

Table No. 6.1- List of chemical used for the work

CHEMICALS	COMPANY
n-Hexane	Molychem, Mumbai
Chloroform	Molychem, Mumbai
Ethanol	Molychem, Mumbai
Methanol	Molychem, Mumbai
Silica gel G	Molychem, Mumbai
Petroleum Ether	Molychem, Mumbai
Ethyl Acetate	Molychem, Mumbai

C. Equipments:-

Table No. 6.2- List of equipments used for the work

EQUIPMENTS	COMPANY
Ultraviolet spectrophotometer	Shimadzu
Weighing balance	Shimadzu
Hot air oven	Bio- Technics India
Soxhlet apparatus	J-Sil
IR	Agilent

2.1 Collection, Authentication And Drying Of Plant Material:-

A) Collection:-

The whole plant of *Coccinia* Grandis was collected in the month of April 2025 from Sangulwadi, Dist Sindhudurg, India.

B) Authentication:-

The plant was identified by (name), Head of department of botany principal (college name and address).

C) Drying:- Drying of plant material was done using shade drying and crushed in an electrical grinder and then powdered.

2.2 Extraction of Plant Material:-

About 2 kg of shade dried plant leaves of Coccinia grandis extracted in soxhlet successively extracted with n-hexane, chloroform, ethyl acetate and methanol. Each extract was evaporated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant leaves. The Consistency and Colour of the extract were noted. All the solvents used for this work were of analytical grade.

% Yield= Weight of extract (g)/ Weight of dry powder(g)×100

Fig no 6.1- Soxhlet extraction apparatus

2.3 Qualitative Chemical Tests[69]

The n-hexane, chloroform, ethyl acetate, methanol extracts of the leaf powder of Coccinia grandis and Orthosiphon stamineus were subjected to qualitative chemical analysis.

2.3.1 Test for alkaloids

The extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with different alkaloidal reagents.

a. **Mayer's** reagent Treated with Mayer's reagent (mixture of potassium and mercuric chloride). Appearance of cream colour precipitate shows the presence of alkaloids.
b. **Dragondroff's** reagent Extract is treated with Dragondroff's reagent (Sodium nitoprusside and iodine). Appearance of orange colour precipitate shows the presence of alkaloids.
c. **Hager's** reagent Extract is treated with Hager's reagent (saturated aqueous solution of picric acid). Appearance of yellow precipitate shows the presence of alkaloids.
d. Wagner's reagent. Extract is treated with Wagner's reagent (Iodine in Potassium iodide). Appearance of reddish brown precipitate shows the presence of alkaloids.

2.3.2 Test for carbohydrates

The minimum amount of the extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

a. **Molisch's** test It was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of violet colour ring at the junction of two layers confirms the presence of carbohydrates.
b. **Fehling's** test The filtrate was treated with 1 ml of Fehling's A and B and heated in a boiling water bath for 5-10min. Appearance of reddish orange precipitate shows the presence of carbohydrates (reducing sugar).

2.3.3 Test for glycosides

a. **Cardiac glycoside**

   Keller-Killani test-

   To 2 ml of extract, glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid were added. Formation of reddish brown colour at the junction of the two liquid layers indicates the presence of cardiac glycosides.

b. **Saponin glycosides**

   Foam test –

   The extract and powder was mixed vigorously with water. Appearance of foam indicates the presence of Saponins.

c. **Coumarin glycosides**

   Methanol extract when made alkaline, shows blue or green fluorescence.

2.3.4Test for phytosterol

1gm of the methanol extract was dissolved in few drops of dry acetic acid; acetic anhydride (3ml) was added and few drops of concentrated sulphuric acid. Bluish green colour confirms the presence of phytosterol.

2.3.5 Test for fixed oils and fats

a. **Filter paper Test:-**

   Various extracts was separately pressed between two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil.

b. **Saponification Test:-**

   Few drops of 0.5N alcoholic KOH is added to a small quantity of extracts along with a drop of phenolphthalein. The mixture was heated for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

2.3.6 Test for tannins and phenolic compounds

Small quantity of various extracts were taken separately in water tested for the presence of phenolic compounds and tannins with

a. Dilute ferric chloride solution (5%) formation of violet colour indicates the presence of phenolic compounds.

b. 1% solution of gelatin with 10%NaCl formation of white precipitate indicates the presence of tannins.

2.3.7 Test for proteins

Various extracts were dissolved in few ml of water and treated with

a. **Millon's reagent** gives the appearance of red colour shows the presence of proteins and free amino acids.

b. **Biuret test:** Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

2.3.8 Test for gums and mucilage

About 10ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

2.3.9 Test for flavonoids

a. With aqueous solution of sodium hydroxide blue to violet colour (Anthrocyanins), yellow colour (Flavones), yellow to orange (Flavonones).

b. With concentrated sulphuric acid yellowish orange colour (Anthrocyanins), orange to crimson colour (Flavonones).

2.3.10 Test for terpenoids Noller's test:

The substance was warmed with thionyl chloride and Tin. Pink color indicates the presence of triterpenoids.

2.3.11 Test for steroids Libermann – Burchard Reaction:

2 ml extract was mixed with chloroform. To this 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. First red, then blue and finally green colour appears.

2.4 Thin Layer Chromatography of methanol extracts of Coccinia grandis

The various methods of separating and isolating plant constituents, thin layer chromatography (TLC) is one of the most powerful techniques used for the separation, identification and estimation of single or mixture of components present in various extracts. Mechanism employed in this reliable technique is adsorption in which solute adsorbs on the stationary phase according to its affinity.

2.4.1. TLC Plates

Precoated silica gel on aluminium plates were used as a stationary phase.

2.4.2 Sample application

The extracts to be analyzed were diluted with respective solvents and then spotted with help of capillary tube just 2 cm above its bottom.

2.4.3 Selection of mobile phase

Solvent mixture was selected on the basis of the phytoconstituents present in each extract. Solvents were analyzed as its order of increasing polarity. Several mobile phases were tried for the separation of maximum components.

2.4.4 Solvent system

Methanol: Ethyl acetate: Water (6:3:1) Rf values were noted down for each selected extracts after elution by using different detecting agents such as Dragondroff's, Ninhydrin, Libermann Burchard, concentrated sulphuric acid and ferric chloride.

2.4.5 Isolation and Characterization of Phytoconstituents Coccinia Grandis by Column Chromatography

Column chromatography is the most useful isolation technique in this the phytochemicals are being eluted by adsorption. The principle involved in this separation is adsorption mainly at the interface between solid and liquid. The components have various degree of affinity towards adsorbent and have reversible interaction to get successful separation. First Low affinity compounds will elute.

2.5 Coccinia Grandis

The methanol extract was subjected to Silica gel column chromatography for the isolation of the phytoconstituents. An appropriate column sized 5cm diameter and 50cm length was used. It was washed with water and rinsed with acetone and then dried completely. Little of pure cotton was placed at the bottom of column with the help of a big glass rod. Solvent hexane was poured into the column upto ¾th level. Methanol extract was mixed with equal amount of graded silica gel until it became free flowing powder. When it reached a defined state it was slowly poured into the column containing hexane solvent with slight movement of stirring by glass rod to avoid clogging. Little cotton was placed on top of silica gel- extract mixture pack to

get neat column pack. The knob at the bottom was slowly opened to release the solvent. The elution was done using hexane, ethyl acetate and methanol in different ratios like Hexane (100%- fraction 1), Hexane: Ethyl acetate (50:50- fraction 2), Ethyl acetate (100%- fraction 3), Ethyl acetate: Methanol (50:50 – fraction 4) and methanol (100%- fraction 5). All the fractions were collected separately and subjected to TLC. The solvents were evaporated by rotary vacuum evaporator. Since there was no yield in the fraction 2 (100%) and very less yield in fractions 1,4 and 5, fraction 3 was selected.

2.6 Spectral Analysis[73-75]

The isolated compounds were analysed to find out the structure by instrumental spectral analysis such as Infra Red spectroscopy.

2.6.1 Infrared spectroscopy

IR spectrum is a vibrational-rotational spectra. For liquid compound Nujol mull method and KBr pellet technique is used for solid compound method is used . It gives information about functional group present in the organic compounds. Bond stretching and bending mechanism is happened when electromagnetic radiation ranging from 500cm^{1} to 4000 cm^{-1} passed through sample. Simanzu Spectrometer was used.

2.7 In-Vitro Urolithiatic Activity

2.7.1 Assessment of in vitro antiurolithic activity of EEBS

Chemicals: Calcium chloride dihydrate, Tris-buffer, Sodium oxalate (Sisco Research Laboratories Pvt. Ltd., Mumbai, India).

Stock calcium chloride and sodium oxalate solutions (respectively 20x10^{-3}M and 1.0x10^{-3}M) were buffered at pH 5.5 with 9 m M sodium di-methyl arsinate and brought to an ionic strength of 0.15 M.

2.7.2 Nucleation assay

Inhibition capacity of plant extract on calcium oxalate (CaOx) crystallization was determined according to the classical method described by Hennequin et al.[76] 1 mL of 0.025 M calcium chloride solution, 2 mL of 0.05 mol/L Tris-buffer, 1 mL of EEBS/ standard compound cystone at various concentrations (10-100 mg/mL) were added to test tube and then 1 mL of 0.025 M sodium oxalate was added. Solution was transferred to a beaker and then constantly stirred at room temperature. Procedure was repeated for six duplicates for each sample. The rate of nucleation was determined by comparing appearance of crystals that reached critical or optically detectable size in the presence of extract and that of control with no extract.[77] The absorbance was recorded at 620 nm and the percentage inhibition was calculated by using the formula:

Percentage inhibition (%) = Abs $_{test}$ /Abs $_{control}$ X 100

2.7.3 Aggregation assay

Aggregation of CaOx crystals was determined by following the method of Atmani et al.[78] The CaOx crystals were prepared by mixing 1 mL of 0.025 M calcium chloride and 1mL of 0.025 M sodium oxalate. Both solutions were then equilibrated at 60ºC in a water bath for 1 hr and then cooled to 37ºC overnight. The formed crystals were harvested by centrifugation for 5 mins and then evaporated at 37ºC. The crystals were used at final concentration of 0.8 mg/mL, buffered with Tris-hydrochloride 0.05 mol/L and sodium chloride 0.15 mol/L at pH 6.5. Experiments were conducted at 37ºC by adding 1mL of EEBS or cystone (standard) at various concentrations (10-100 mg/mL). The rate of aggregation was estimated by comparing turbidity in

presence of EEBS/standard with that of control. The absorbance at 620 nm was recorded spectrophotometrically. The percentage inhibition rate (Ir) was calculated by the following formula:

Percentage inhibition (Ir) = 1- (Turbidity $_{test}$ / Turbidity $_{control}$) X 100

% IN = [1 - (OD_{sample}/$OD_{control}$)]x100

Where OD_{sample} is the optical density of EECB or cystone, $OD_{control}$ is optical density without EECB or cystone

2.7.4 Aggregation assay

The effect EECB on the nucleation of crystals was analysed at concentrations of 10, 50, 100, 200, 400, 600, 800, and 1000 µg/ml. The crystals of calcium oxalate were prepared by adding equal volume of CaCl2 and Na2C2O4 at 0.1 mol/L; heated up to 60°C for 3 h and allowed to stand overnight at 37°C. The calcium oxalate crystals were separated by centrifugation at 2000 rpm and dried at room temperature. Buffer (pH 6.5) containing Tris 50 mmol/L and sodium chloride 150 mmol/L were used as solvent to prepare 0.75 mg/ml calcium oxalate crystals. 9 ml of calcium oxalate crystals solution was mixed with 1 ml of EECB and incubated for 60 min at 37ºC. The distilled water was used as blank and Cystone as a standard. The OD was taken at 620 nm by a UV-Visible spectrophotometer. The aggregation inhibition (% AI) was calculated by the following formula:

% AI = [1 - (OD_{sample}/$OD_{control}$)]x100

Where ODsample is optical density with EECB or cystone, ODcontrol is optical density without EECB or cystone.

2.7.5 Image analysis of calcium oxalate (CaOx) crystal morphology (Microscopic assay)

Incubation of metastable solutions of calcium chlorie and sodium oxalate resulted in the formation of CaOx crystals. The harvested crystals were centrifuged and placed on a petriplate glass slide. Various concentration of EEBS (20, 40, 80, 160 µg/mL) and control were then applied directly to the crystals. Change in structure of CaOx crystals were compared with the control by observing under microscope after 30 mins to determine how crystals were dissolved by extract. Crystal size was observed under Leica stereo zoom dissecting microscope with digital imaging system at 4X and the photographs were taken.

2.8 *In-Vitro* Nephroprotective Study

2.8.1 Strains and growth conditions

The following Gram-negative and Gram-positive strains were used in this study: *Escherichia coli* strain GC4468 (F^- Δlac U169 *rpsL*) and *Staphylococcus aureus* strain ATCC25923[9]. Overnight cultures were grown in a shaking water bath at 200 rpm and 37°C respectively.

For experiments, the overnight cultures were either diluted 200-fold in LB medium and grown to mid-log phase in a shaking water bath at 200 rpm and 37°C or diluted and used as stationary phase cultures. When necessary, cells were thoroughly washed with PBS to remove traces of medium and resuspended to OD_{600nm} = 0.5 or 0.1 in PBS containing 0.2% glucose. Experiments were performed in 96-well plates. Aliquots (100 µl/well) of cell suspensions were transferred into triplicate wells, and tested compounds were added. Stock solutions of tested compounds were prepared in PBS and filter-sterilized.

M9CA medium consisted of M9 salts, prepared by dissolving 6 g Na_2HPO_4, 3 g K_2HPO_4, 1 g NH_4Cl, and 0.5 g NaCl in 1 liter of distilled water. To 100 ml of M9 salts, 1 ml of each 0.2 M MgSO4, 20% glucose, casamino acids (Difco) to 20%, and 50 µL of 0.2 M $CaCl_2$, autoclaved separately were added. Immediately before use, filter-sterilized solutions of pantothenic acid and thiamine were added to a final concentration of 3 mg/L. Individual amino acids (Sigma-Aldrich) were added to phosphate-buffered saline (PBS) (Thermo Fisher Scientific) supplemented with 0.2% glucose or to M9 salts supplemented with 0.2% glucose. Since both media produced similar results, only data obtained in PBS/glucose are presented.

2.8.2 MTT assay

MTT reagent was prepared by dissolving 25 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich) in 5 ml PBS. Unless otherwise specified, 10% SDS in 10 mM HCl was used for solubilization of the formazan crystals. Other tested solubilization solvents were 10% SDS dissolved in 50 mM phosphate buffer, pH 7.4, dimethyl sulfoxide (DMSO), ammonia-containing DMSO and isopropanol. To improve solubility, half of the wells containing identical samples were first treated with SDS dissolved in distilled water (final concentration in wells 5%) or solubilized with organic solvents containing 5% SDS.

Ten µl of MTT reagent were added to all wells and plates were incubated for 30 min on a thermostatic shaker at 37 ºC and 200 rpm in the dark. After 30 min, 100 µl of SDS HCl reagent, SDS in phosphate buffer, ammonia-DMSO, DMSO or isopropanol, with and without SDS were added per well and plates were incubated for 1 h at room temperature in the dark. In cases incomplete formazan dissolution was detected, the time of incubation was extended to 3 hours. The absorbance of each well at 560 nm and 700 nm was measured and absorption spectra of the wells were recorded using a microplate reader.

Or

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using MEM containing 10% PBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 m l of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37º C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24h interval. After 72 h, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37º C in 5% CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

2.9 Molecular Docking Study

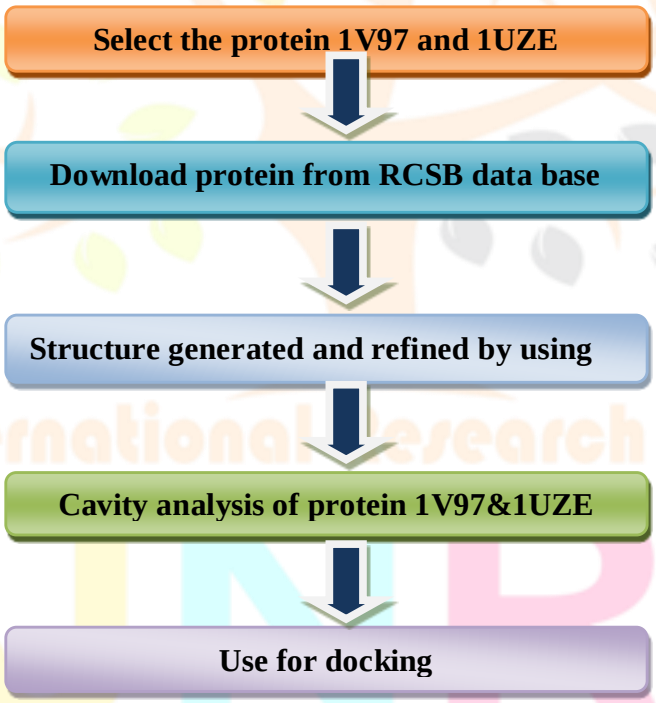
Molcular dockeeng is a computational technique that expects the obligatory affinity forminor molecules to an target protien binding. It plays an essential part of medication development and research by providing insights into the relationship between a drug candidate and its target protein. Through molecular docking, researchers can identify potential drug intrants that have high binding affinity to the target protein, which can lead to the

development of more effective and specific drugs. It also helps in considerate the mechanism of action of drugs and predicting their pharmacological properties. . In reality, it is not a stand-alone method; rather, it is typically incorporated within a workflow that includes many experimental and in silico methods. A number of research teams concentrate on assessing the effectiveness of different docking algorithms or on enhancing the scoring functions once experimental testing has been completed. These endeavors have the potential to provide significant direction in selecting the approach for a certain target system.[79-80]

2.9.1 Protein Preparation

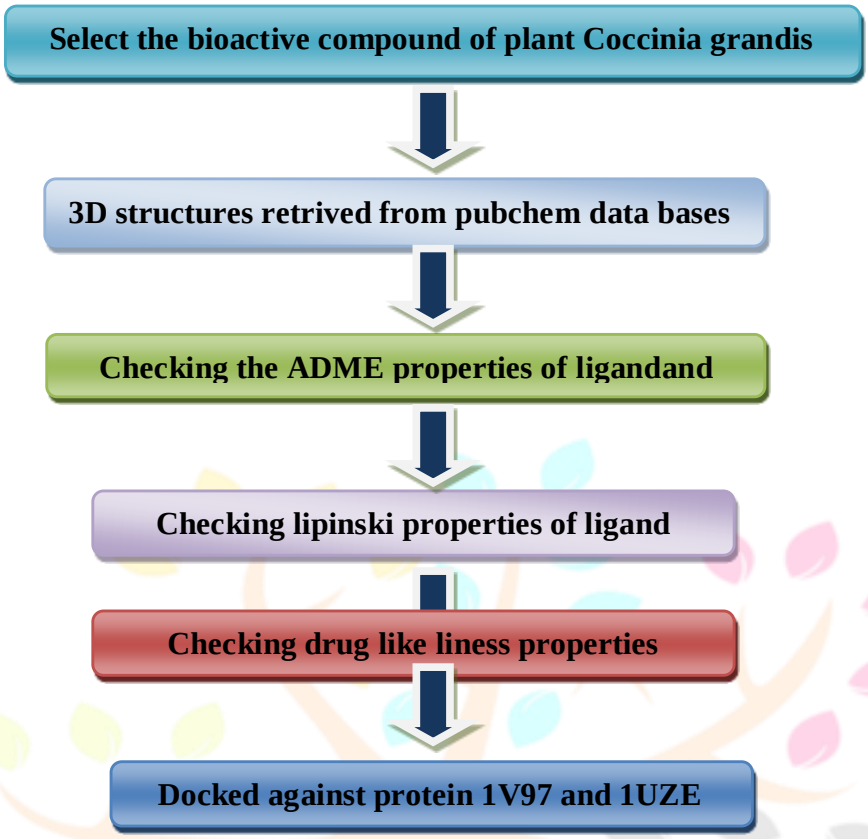
The protein data bank was used to get the pdb format for the crystal structures of the following: human testicles angiotensin I-converting enzymes (PDB id: 1UZE) and the compound of the anti-hypertensive medication enalapril. The crystal structure of the animal Cow's milk a substance bound version of xanthine dehydrogenase FYX-051 (PDB id: 1V97). After that, the structure was created and improved utilizing the Bioviaa Discovery Studio software 2021. All waters were deleted, charges and bond orientations were allocated, and hydrogen molecules were put in to the heavier atoms.

2.9.2 Preparation of protein flowchart



2.9.3 ligand Preparation

1) Preparation of ligand flowchart



2.9.4 Ligand Preparation

We obtained the compounds from the Pubchem databases. Betasitosterol, Cucurbitacin, kaempferol, Thiamine, Ferulic Acid, Quercetin, ombiun, Ascorbic acid, trans p coumaric acid, Carbonic Acid, Niacin with their 3D structures.

2.9.5 Drug likeness study of ligand

The term "druglikeness" refers to a qualitative notion in drug design that describes how "drug similar" a material is in relation to variables such as bioavailability. Checking for adherence to The Five Lipinski Rules, that addresses quantity of polar groups, hydrophobicity, & molecular weight, is a conventional technique of assessing druglikeness. An agent similar to a drug contains 20–70 atoms, The molaar refractivity of 40–130 and a molecular mass of 160–480 g/mol. The amount of space and molecular weight of the molecule are related to these characteristics. In theory, the coefficient of partitioning (log P) has a logarithmic value between -0.4 and 5.6. Known as the Pfizer rule of five, Lipinski's principle of five, or simply the principle of 5 (RO5), is a generic guideline. for assessing druglikeness. It is crucial to ascertain a drug's efficacy or whether a chemical compound exhibiting a particular biological or pharmacological action possesses characteristics that would likely make it an orally therapeutic agent in humans.

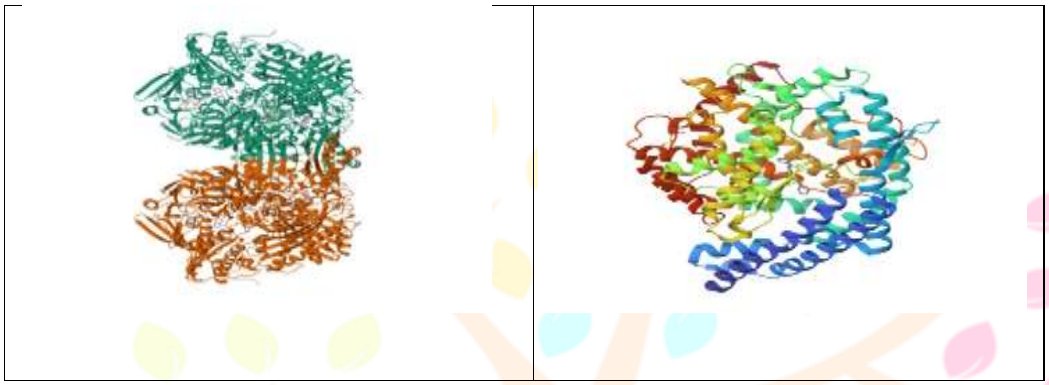
2.9.6 ADMET properties of ligands

Checking the ADMET studies of each component is required for in-silico screening of ligands. ADMET is an abbreviation intended for distribution, absorption,metabolism, elimination, and toxicity, and it supports in the selection of small compounds for molecular docking studies based on basic guidelines.It is utilized in the drug evolution process to test the applicability of Lipinski and ADMET properties of ligand molecules to act as drug molecules. Swiss ADME, publicly accessible software, was used to determine the properties of *Coccinia*

grandis bioactive components such as absorption, metabolism, excretion, and distribution.Lipinski's rule of 5 is based on molecular mass<500, amount of donors for hydrogen bonds <5, quantity of acceptors for hydrogen bonds <10, and log P<5. As a result, Swiss ADME was used to filter the best-docked compounds. Log BB, total CNS activity, caco-2, skin permeability, GI adsorption, and other properties were also predicted.[81-82]

2.9.7 Molecular docking studies

The major bioactive compounds of *Coccinia grandis*were docked against The animal Cow's milk a substance FYX-051 bounded form of xanthiinedehydroginaze (PDB id: 1V97), and the Combination of the human testicles angiotenseen I-converting enzyme (PDB id:1UZEs) with the anti-hypertensivea medication enalapril utilizing Pyrx software 0.8.



(a) **(b)**

Three-dimensional structure of (a) The animal cow's milk substance FYX-051, a bounded form of xanthiinedehydroginase (PDB id: 1V97), and (b) the combination of the human testicles and angiotensin-converting enzyme (PDB id: 1UZEs).

2.9.8 DOCKING INTERPRETATION

The affinity of binding for ligands with the receptor was identified by using various elements such as binding affinity (in kcal/mole), hydrogen bonds, van der Waals forces, pi-alkyl interactions, pi-sigma bonds, pi cations, and anion bonds. Ligands with stronger bond interactions were considered significant for the present investigations. Based on comprehensive analyses such as drug-likeness properties, Lipinski's rule, ADMET properties, Protox 2, pkCSM, SwissADME, the number of lead molecules with high binding affinity, favorable drug-like properties, and the development of potential antiurolithiatic and nephroprotective properties.

3. RESULTS AND DISCUSSION:-

7.1 Extraction and Phytochemical analysis:-

For *Coccinia grandis* leaves, the % yield with increasing extractive values is recorded. In table no: 1.

Table no: 3.1 The proportion produce of successive extracts of the leaf of Coccinea grandees.

Sr.No	Extract	Colour	Physical Nature	Percentage yield (w/w)
1	n-Hexane	Green/Sticky mass	Waxy Semisolid	1.8
2	Chloroform	Green/Sticky mass	Semisolid	2.0
3	Ethyl Acetate	Brownish green solid	Solid	3.4

4	Methanol	Brownish green solid	Solid	7.9

Several polarity phytoconstituents were extracted using a soxhlet device and solvents such as nhexane, chloroform, ethyl acetate, and methanol in order to perform a phytochemical analysis on the leaveCoccinia grandis powder. The specific polarity & solubility characteristics of the metabolites inside the plant were revealed by successive extractive values. Comparing methanolic extract to other Coccinia grandis extracts, the former had a high extract yield of 7.9% w/w.

3.2 Chemical Analysis by Qualitative

Qualitative chemical examination of plant based constituents in Coccinia grandis leaf extracts, as shown in Table 2.

Table no: 3.2 The phytochemical examination of *Coccinia grandis*

Sr.No	TEST	n-Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	-	+	-
2	Carbohydrate	-	-	+	-
3	Glycosides	-	+	-	+
4	Phytosterol	-	-	-	+
5	Fixed oils and Fats	-	-	-	-
6	Tannins	-	-	-	-
7	Phenols	-	-		+
8	Proteins	-	-	+	-
9	Gums and Mucilages	-	-	-	-
10	Flavonoids	-	-	-	+
11	Terpenoids	-	-	-	+
12	Steroids	-	+	-	-
13	Saponins	-	-	+	+

Notea: A good outcome is shown by a +ve, while a negative result is indicated by a -ve.

Using various chemical reagents, a preliminary qualitative phytochemical study of Coccinia grandis was first carried out to determine the many kinds of phytoconstituents & that were present within every excerpt. In the chloroform extract, steroids and glycosides were detected.

.3 Thin Layer Chromatography Method

Table no: 3.3 The TLC analysis of Coccinia grandis methanol extracts.

S.No	Name of the Extract	Solvent system	No of spots	Rf Values
1	Methanol (CGM)	Methanol : Ethyl acetate: Water (6:3:1)	01	0.33

Fig. No. 3.1. TLC of isolated compound

3.4 UV Spectrum:-Fraction in Methanol

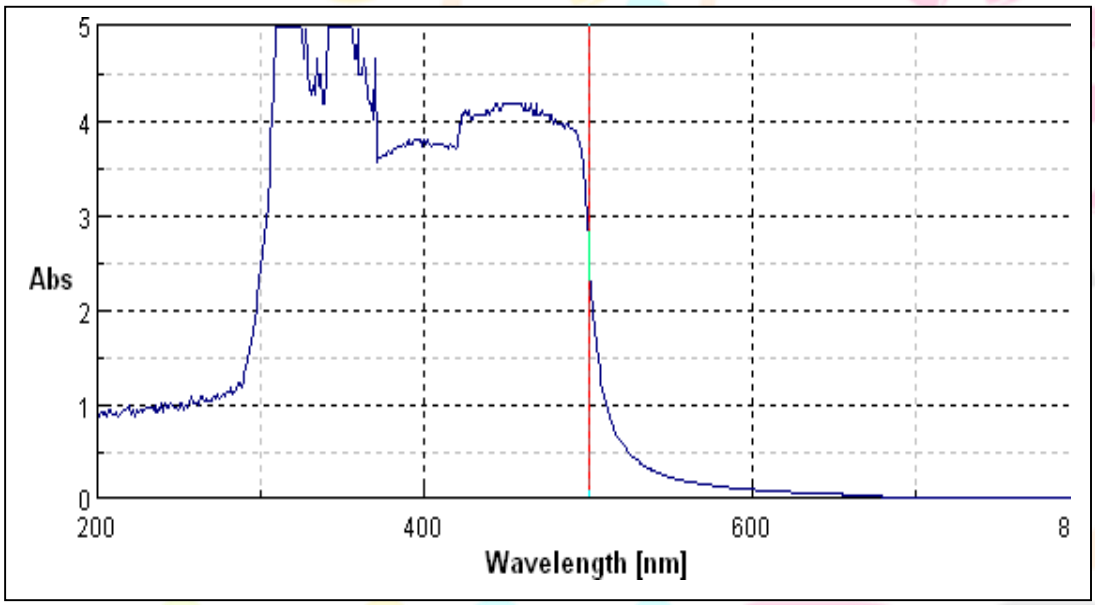


Fig. No. 3.3.The UV spectrum showed characteristic bands of fraction in methanol at

The UV spectra of isolated extract from *Coccinia grandis*was performed using methanol. The UV spectrum showed characteristic bands of fraction in methanol at The UV spectra of standard showed one characteristic peak at

3.5 FTIR Spectrum

Table no: 7.4 FTIR Spectrum analysis

Sr.No.	Peak Values	Bonds	Functional groups
1	2952.75	O–H stretch, H–bonded	Alcohols, Phenols
2	2921.35	C–H stretch	Alkanes
3	1493.22	C–C stretch (in–ring)	Aromatics
4	1378.31	C–O stretch	Alcohols
5	842.46, 698.95	C–H “oop”	Aromatics

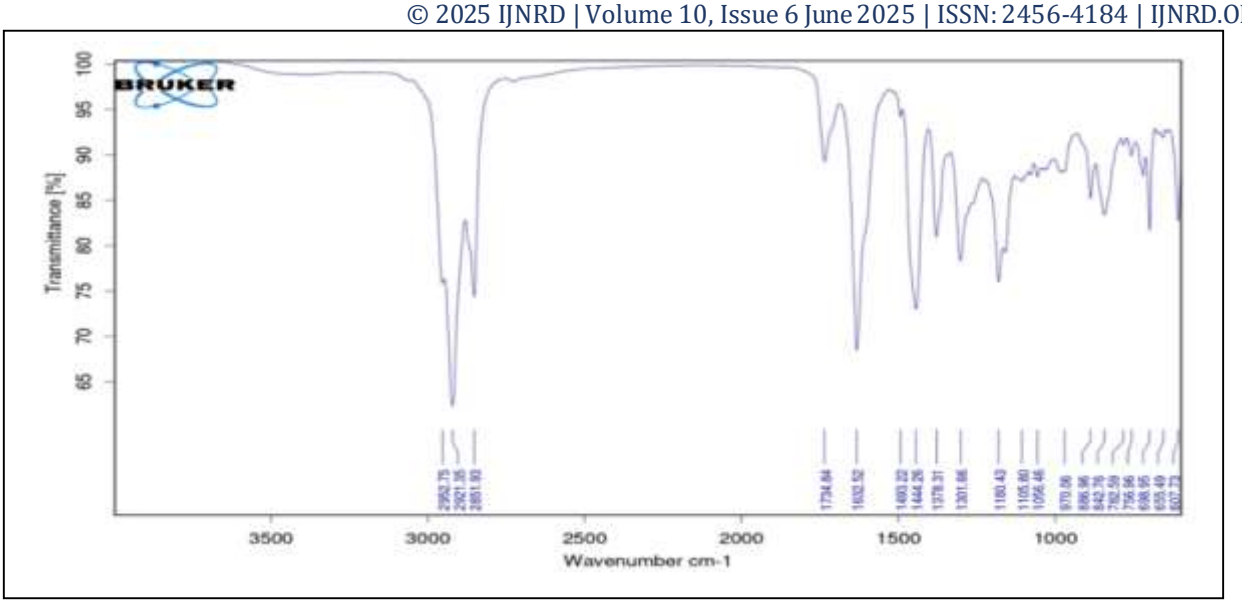


Fig. No. 4. FTIR Spectrum of *Cocciniagrandis* extract

3.6 *In-Vitro* anti-Urolithiatic

3.6.1 Crystal Aggregation assay

COM crystal:

Table no: 3.5- Std drug Cystone tab conc.(1mg/ml)

Std drug Cystone tab conc.(1mg/ml)	concentration	O.D	% inhibition
Control		0.22	
5 min	10	0.10	54.54
	20	0.11	50.00
	30	0.10	54.54
	40	0.12	45.45
	50	0.12	45.45
10 min	10	0.09	59.09
	20	0.10	54.54
	30	0.09	59.09
	40	0.07	68.18
	50	0.08	63.63
15 min	10	0.09	59.09
	20	0.07	68.18
	30	0.07	68.18
	40	0.08	63.63
	50	0.07	68.18
20 min	10	0.06	72.72
	20	0.07	68.18
	30	0.06	72.72
	40	0.07	68.18
	50	0.06	72.72
25 min	10	0.05	77.27
	20	0.06	72.72
	30	0.04	81.81
	40	0.05	77.27
	50	0.04	81.81

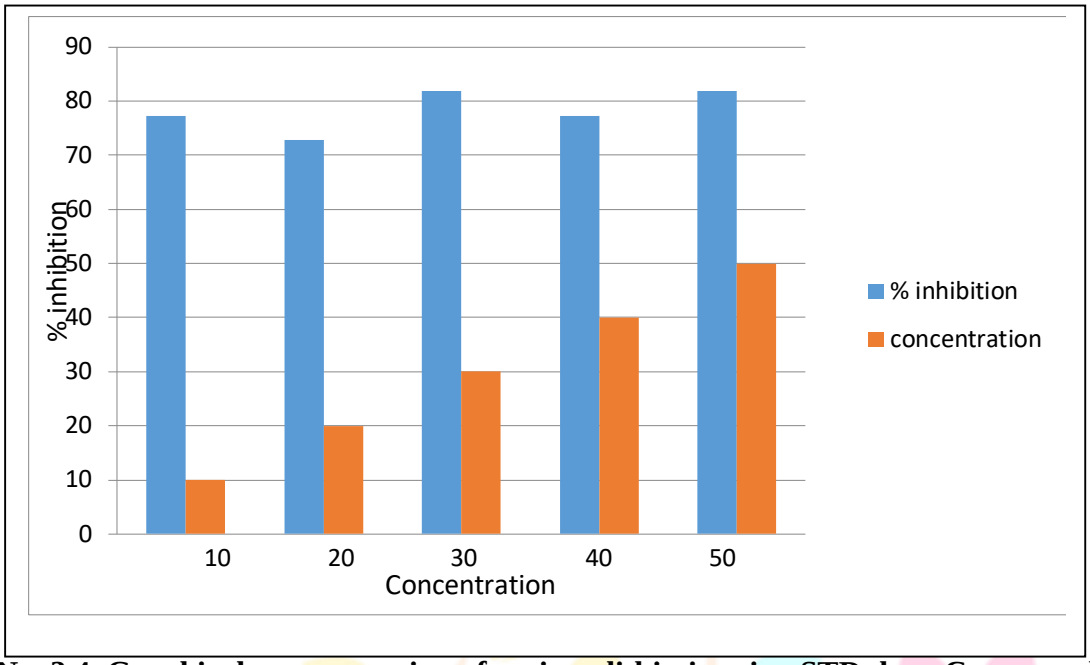


Fig. No. 3.4. Graphical representation of anti-urolithiatic using STD drug Cystone tab conc.

Table no: 3.6- Sample *Coccinia grandis extract*

Sample *Coccinia grandis extract*	**concentration**	**O.D**	**% inhibition**
Control		0.22	
5 min	10	0.21	4.51
	20	0.20	9.09
	30	0.21	4.51
	40	0.20	9.09
	50	0.20	9.09
10 min	10	0.19	13.63
	20	0.18	18.18
	30	0.18	18.18
	40	0.17	22.72
	50	0.17	22.72
15 min	10	0.16	27.27
	20	0.15	31.81
	30	0.15	31.81
	40	0.16	27.27
	50	0.16	27.27
20 min	10	0.14	36.36
	20	0.13	40.90
	30	0.13	40.90
	40	0.12	45.45
	50	0.12	45.45
25 min	10	0.10	54.54
	20	0.09	59.09
	30	0.10	54.54
	40	0.10	54.54
	50	0.09	59.09

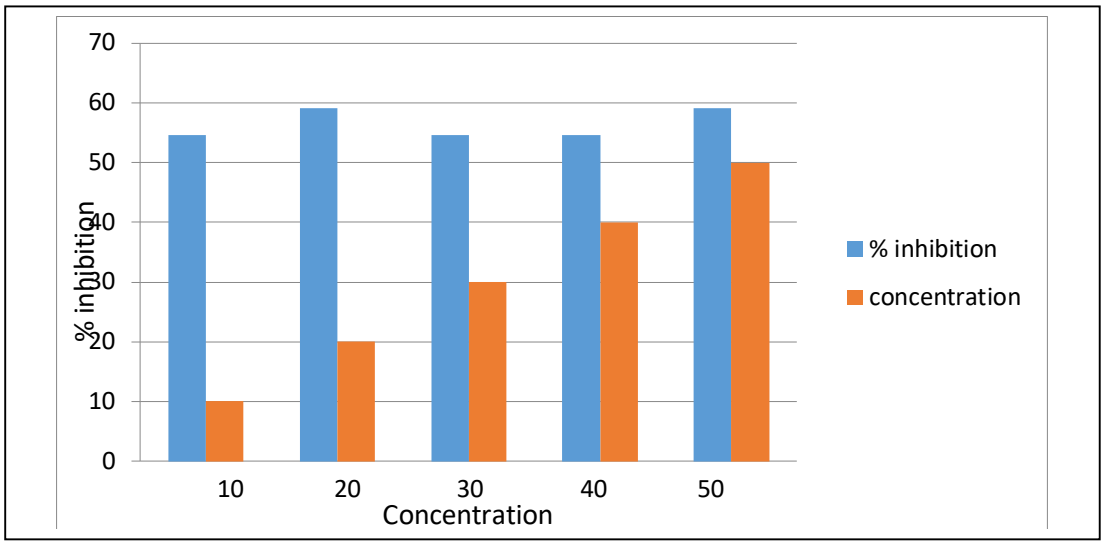


Fig. No. 3.5. Graphical representation of anti-urolithiatic using methanolic extract of *Coccinia grandis*

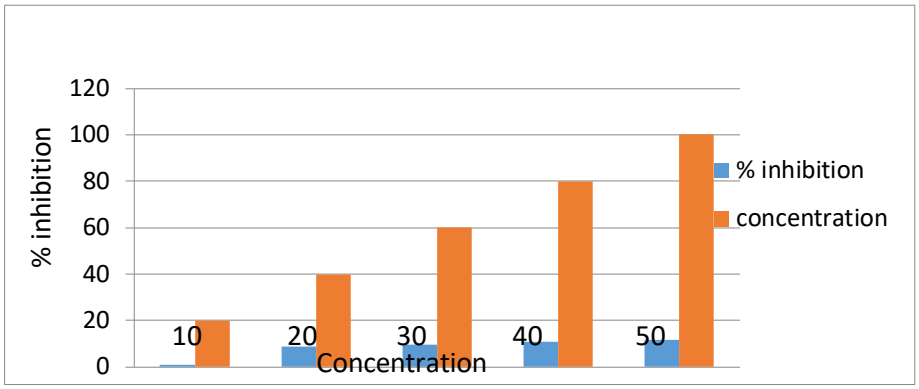


Fig. No. 3.7. Sample *Coccinia grandis extract* different conc.

Conclusion- As compared to standard the given Sample–showed moderated in vitro anti urolithiasis activity

3.7 Nephroprotective Assay

3.7.1 NRK 52 E Cell line (Normal Kidney Cell Line)

Table No.7.7-Effect of compound against NRK52E Cellline

SR NO	AMPLE ODE	Conc.(µg/ml)	OD			Mean	% Of Inhibition	% of Viability	IC50 (µg/ml)
1	Control		1.532			-	-	-	-
2	Standard	**20**	1.453	1.452	1.454	**1.453**	**5.15%**	**94.84%**	
	(5,Flurour -acil)	**40**	1.397	1.396	1.395	**1.396**	**8.87%**	**91.13%**	
		60	1.386	1.387	1.388	**1.387**	**9.46%**	**90.54%**	**NE**
		80	1.369	1.368	1.369	**1.368**	**10.70%**	**89.3%**	
		100	1.350	1.352	1.350	**1.350**	**11.87%**	**88.12%**	
3	CG1	**20**	1.370	1.371	1.372	**1.371**	**10.50%**	**89.5%**	
		40	1.342	1.341	1.340	**1.341**	**12.46%**	**87.54%**	
		60	1.287	1.286	1.285	**1.286**	**16.05%**	**83.95%**	**NE**
		80	1.260	1.262	1.260	**1.260**	**17.75%**	**82.25%**	
		100	1.215	1.214	1.213	**1.214**	**20.75%**	**79.25%**	

***NE-Not Evaluable**

Fig. No. 3.8. Graphical representation of Nephroprotective Standard

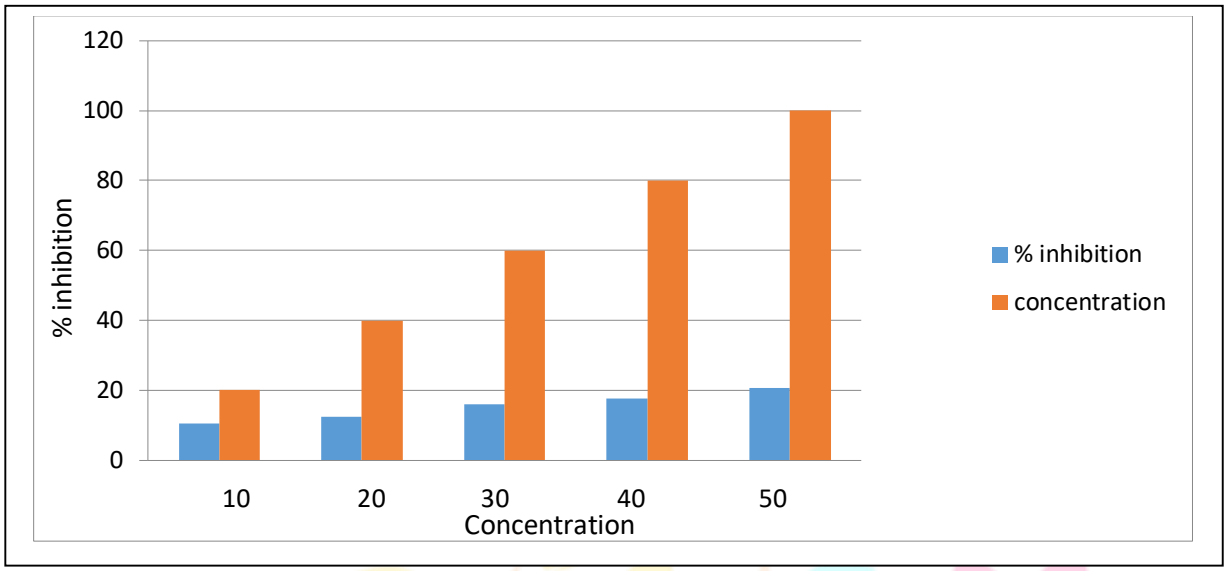


Fig. No. 3.9. Graphical representation of Nephroprotective methanolic extract of *Coccinia grandis*

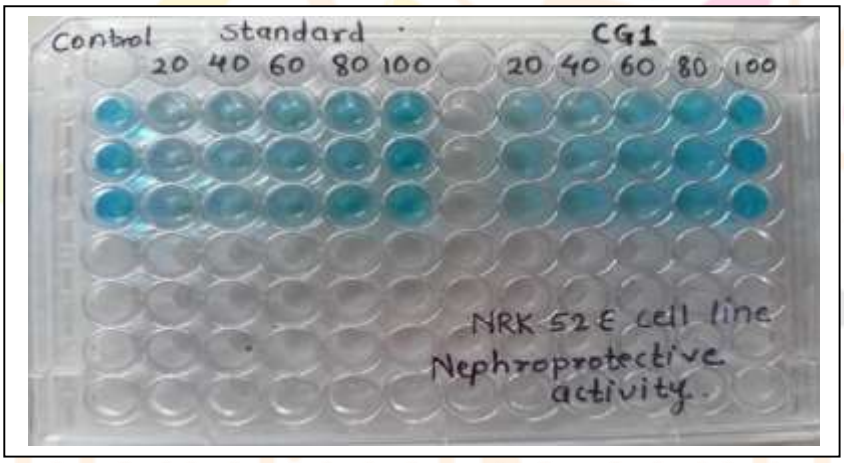
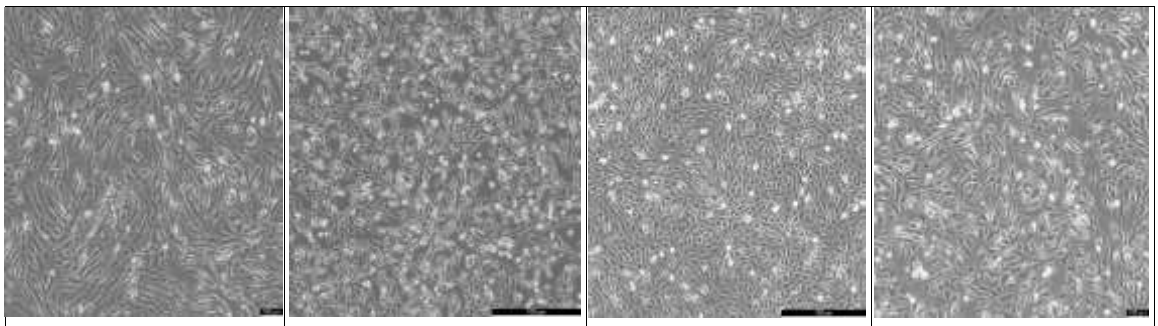


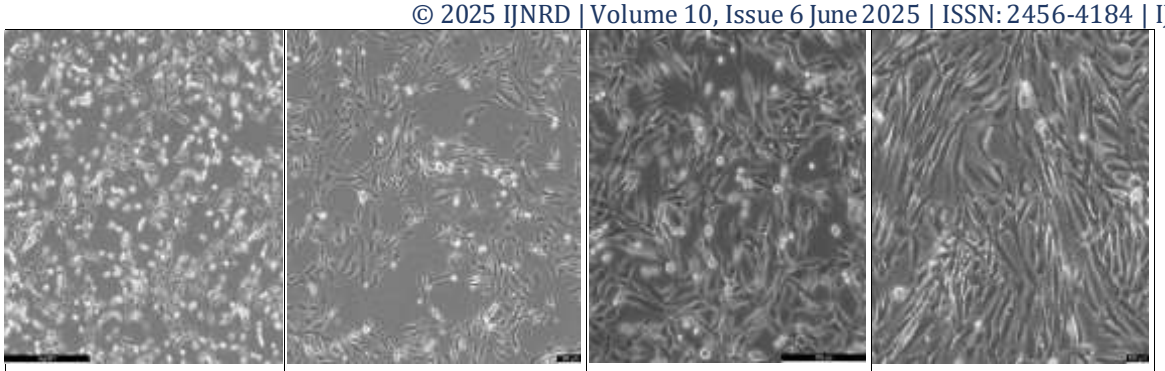
Fig. No. 11. NRK S2E cell line nephroprotective activity

Conclusion:

At the different Concentrations Sample Code **CG1** shows low percent of inhibition and against NRK 52E (Kidney Cells) cell line versus a typical medication 5FU. On basis of percent of viability we can conclude that the sample CG1 may not harm the normal kidney cell lines and it may protect the kidney from the harm organ damages.



20 LBG1	20 5FU	40 5FU	40 LBG1



60 5FU	60 LBG1	80 5FU	80 LBG1

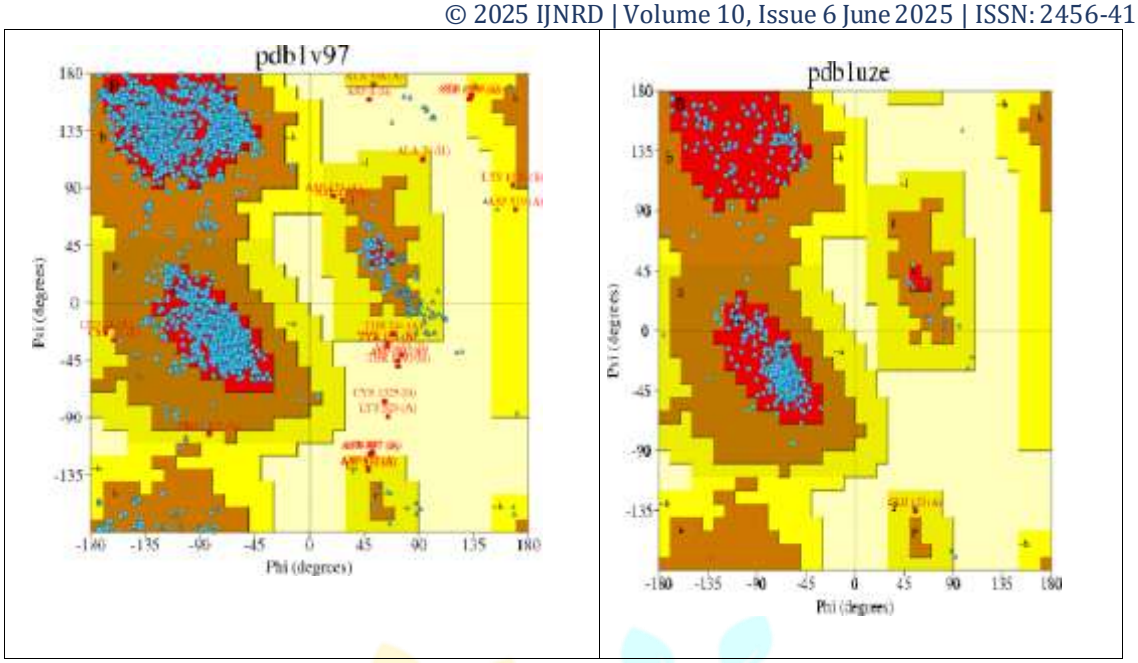
Fig. No. 12. Different Concentrations Sample Code CG1 shows low percent of inhibition and against NRK 52E (Kidney Cells) cell line versus a typical medication 5FU.

3.8 *In-Silico* Study

Coccinia grandis plant contains bioactive compounds like Betasitosterol, Cucurbitacin, kaempferol, Thiamine, Ferulic Acid, Quercetin, ombiun, Ascorbic acid, trans p coumaric acid, Carbonic Acid, Niacin, demonstrating ligand interactions. The rule of 5, Lipinski's rule of 5, helps identify bioactive compounds with molecular docking and drug likeliness. This helps in developing new methods for using phytomedicines in medication development. Lipinski's rule of 5, which includes Log P (<+5), molecular mass (<500), 3. The quantity of donors of hydrogen bonds (<5) 4. There are less than ten hydrogen bond acceptors. 5. 40–130 molar refractivity. Molecular docking results lead to the development of novel drugs against drug targets based on a structure based drug deceitful strategy. The best binding conformation of inhibitors to enzymes was identified with the lowest energy conformation by molecular docking. Protein-ligand complexation gives plenty of pieces of information including hydrogen bonds, lipophilic interactions, π-π interactions from the protein-ligand interaction profile.

3.8.1 Investigation of selected protein

Investigation of protein 1V97 and 1UZE is done by using PDB sum software with the help of Ramchandran plot procheck analysis. According to Ramchandran plot statistics for 1V97 protein the most favoured regions is 88.7%, additional allowed region is 10.3% generally allowed region is 0.6% and disallowed region is 0.4% which are shown in fig. A. Again similarly according to Ramdrachandran plot statistics for 1UZE most favoured regions is 94.3%, additional allowed region is 5.5% generally allowed region is 0.2% and disallowed region is 0.0% which are shown in fig. B.



A) 1V97 **B) 1UZE**

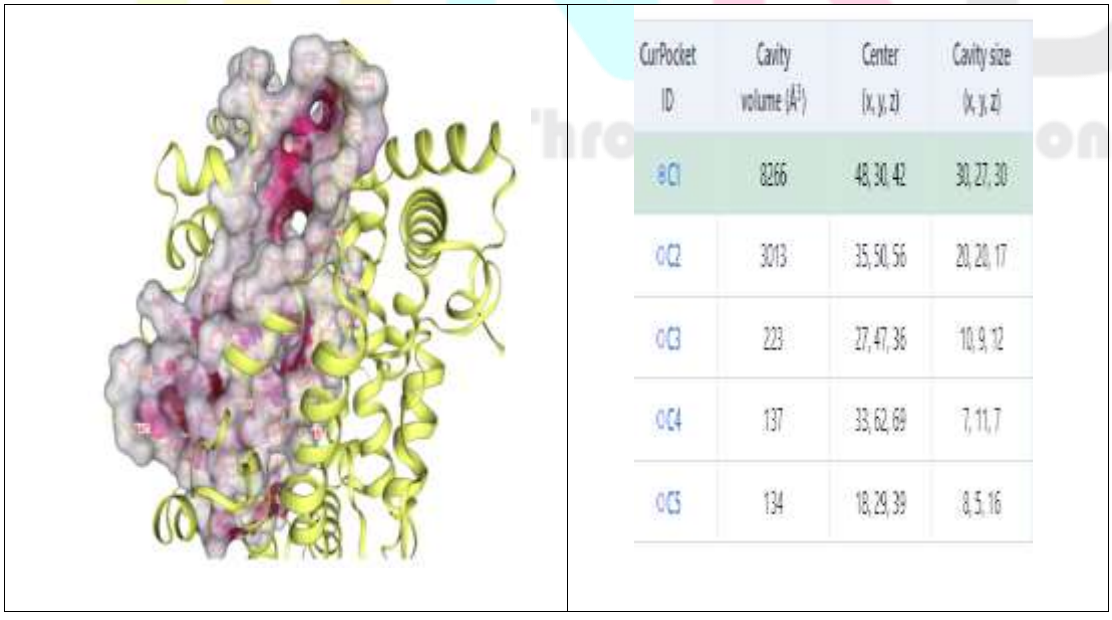
Fig. No. 13. Ramchandran plot of A) 1V97 and B) 1UZE

3.8.2 Cavity identification

Cavity identification of 1V97 and 1UZE protein is done by using CB-Dock 2 software is a potent technique for investigating the binding sites of ligands and the binding positions of receptors.

CurPocket ID	Cavity volume (Å^3)	Center (x, y, z)	Cavity size (x, y, z)
●C1	5802	135, 21, 34	30, 21, 30
○C2	4526	120, 2, 35	20, 26, 24
○C3	4429	120, 38, 38	20, 27, 25
○C4	3476	107, 2, 59	19, 23, 21
○C5	3260	108, 38, 10	18, 23, 19

Fig. No. 14. A. Cavity detection of protein 1V97



CurPocket ID	Cavity volume (Å^3)	Center (x, y, z)	Cavity size (x, y, z)
●C1	8266	48, 30, 42	30, 27, 30
○C2	3013	35, 50, 56	20, 28, 17
○C3	223	27, 47, 36	10, 9, 12
○C4	137	33, 62, 69	7, 11, 7
○C5	134	18, 29, 39	8, 5, 16

Fig. No. 14. B. Cavity detection of protein 1UZE

All data obtained from molecular docking of bothproteins with ligands are depicted in Table.10

Table no: 10. Lipinski properties of bioactive compounds from*C.grandis* leaves

Sr. No.	Compound Name	Molecular Weight (g/mol)	Log P	H-bond Donor	H-bond Acceptor	Molar Refractivity
1	Betasitosterol	414.71	4.79	1	1	133.23
2	Cucurbitacin B	558.70	1.46	3	8	150.94
3	Kaempferol	286.24	1.47	4	6	76.01
4	Thiamine	265.35	-1.60	2	3	73.12
5	Ferulic Acid	194.18	1.62	2	4	51.63
6	Quercetin	302.24	1.63	5	7	78.03
7	Ombium	330.29	2.83	3	7	51.63
8	Ascorbic Acid	176.12	0.39	4	6	35.12
9	Trans p-Coumaric Acid	164.16	0.95	2	3	45.13
10	Carbonic Acid	62.02	-0.22	2	3	10.65

Table no: 11. ADME properties of various bioactive compounds

Sr no	Compound name	Formula	Distribution	Absorption				Toxicity
			BBB Pearmiability	GI Absoⁿ	Human Intestinal absorption	Skin pearmiability	CACO-2Pearmiability	Toxicity predicted LD 50
1	Betasitosterol	C29H50O	0.781	Low	94.464	-2.783	1.201	2.552
2	Cucurbitacin B	C32H46O8	-1.003	Low	89.52	-3.496	0.588	2.381
3	kaempferol	C15H10O6	-0.939	High	74.29	-2.735	0.032	2.449
4	Thiamine	C12H17N4O5	-0.368	High	100	-2.792	0.867	2.672
5	Ferulic Acid	C10H10O4	-0.239	High	93.685	-2.72	0.176	2.282
6	Quercetin	C15H10O7	-1.098	High	77.207	-2.735	-0.229	2.471
7	Ombiun	C17H14O7	-1.089	High	87.47	-2.735	0.402	2.272
8	Ascorbic acid	C5H8O6	-0.985	High	39.154	-2.955	-0.255	1.063

9	transp coumaric acid	C9H8O 3	-0.225	High	93.494	-2.715	1.21	2.155
10	Carbonic Acid	CH2O3	-0.428	High	83.064	-2.737	1.12	1.439
11	Niacin	C6H2O N4O6	-0.323	High	94.099	-2.735	1.17	2.24

Table no: 12 Molecular docking result examination of bioactive substances of *coccinia grandis* against the protein 1V97

Compound Name	Binding affinity (kcl/mol)	Conventional H- bond Interaction	Pi Sigma Interaction	Pi Alkyl Interaction	Van- der -Waals Interaction	Other Interaction
Betasitosterol	-8.9	-	-	ALA424, ALA 432, LYS1172 TRP336, ARG427	PHE 421, GLN423, THR435, GLN333, HIS1171, ASP1165, LEU547, LYS518, LEU548, LYS995, PHE549, GLN550, ASP1170, GLU332, ASP 430	-
Cucurbitacin B	-8.6	GLY260, THR354, GLU263, THR262	-	-	ASN261, VAL 345, ALA346, VAL259, LEU257, VAL258, ILE264, ILE353	-
Kaempferol	-9.1	GLU263, SER 347, TRP336, LYS422	-	ALA346, ALA338	ASN351, THR354, ILE431, THR262, GLY350, LEU305, ASP360, ILE358, ASP 429, ASP430	Other Pi-Pi Stacked-PHE337
Thiamine	-7.2	SER 69, SER 307, SER 306,ASN 130	THR 126	-LYS 340	SER 344, TLE 66, ALA 304, LYS 310,HIS 67 ,GLU 188,GLN 131, LEU 127, SER 123,PHE 68	-

Ferulic Acid	-7.4	LEU 404, GLY 260	LEU 257	ILE 353 ,VAL 259	- ILE 403, ALA 301, LEU 287, GLY 349, VAL 258, ALA 346. ASN 261, GLU 263, THR 354 ,ILE 264, LYS 256	Other C-H bond- GLY 350
Quercetin	-10.1	ALA 301	-	LEU 257, ILE 353	GLY 349, LEU 404, LEU 398, LYS 249, ILE 403, VAL 259, GLY 350,SER 347,ALA 302	-
Ombiun	-8.7	-	-	-	SER 765, LYS 792, VAL 764, ARG 790, PHE 763, TYR 592,ILE 596, CYS 593, ARG 793	Other C-H bond- VAL 791
Ascorbic acid	-6.3	THR 354, THR 262, GLY 260,SER 347	-	-	ILE 264, GLU 263, ASN 261, ALA 346, VAL 34S ,VAL 259, VAL 258, GLY 350	-
trans p coumaric acid	-6.8	GLN 561, LEU 1243, LEU 1244, GLY 1183	-	-	PRO 1188, PHE 560, CYS 1247, ASP 1246,GLY 574	Other C-H bond- SER 1185, ARG 1245
Carbonic Acid	-3.8	LEU 219, TRP 298	-	LEU 216	LYS220, VAL222, PRO 224, ILE 284, PRO 285, LYS 57, TYR 58	Other- Pipi T shaped- HIS 82C-H bond- ARG 60
Niacin	-5.7	ALA 301	-	-VAL 259, LEU 257	ILE 403, LEU 404, GLY 349, ALA 302, SER 347 GLY 350, VAL 258, LEU 398 LYS 249	-

Table no: 13 Moleculardocknig result examination of bioactive substances of *coccinia grandis* against the protein 1V97

Compound Name	Binding affinity (kcl/mol)	Conventional H- bond Interaction	Pi Sigma Interaction	Pi Alkyl Interaction	Van- der - Waals Interaction	Other Interaction
Betasitosterol	-9.3	GLU 411	-	VAL518,LEU81, LEU140 , LEU139	GLU143,TYR69, ASN70, ASN66, VAL351,ASN136, ASN85 ARG124, SER516, TRP357, SER355, TYR523	-
Cucurbitacin B	-10	ILE 204, ASN211, GLU124	-	-	ALA 207, TRP220, TYR135, LEU139, SER219, ALA208	-
Kaempferol	-8.2	ASP415, TYR520, LYS511, GLN281, ASP377	VAL380	ALA 354	GLN369, GLU162, HIS353, HIS513, PHE457, TYR523, PHE527, HIS383	-
Thiamine	-6.9	TYR 62, SER 355	-	- LEU 82, LEU 139,LEU 81, LEU 140	TYR 69, VAL 351, TRP 357 ,GLU 143 ,ASN 70, PHE 512, VAL 518 ,ASN 85,ASN 136	Other C- H bond-LYS 368, SER 516
Ferulic Acid	-6.5	ARG 124, SER 517	-	ILE 204, ALA 207	LEU 139, TYR 135, SER 219- TYR 213,ASP 121,ALA 208	Other Pi-anion – GlU123 Amide-pistacked – TRP 220
Quercetin	-8.3	THR 301, ASP 453	-	LYS 449, LEU 375	THR 302, ASP 300, MET 299, LEU 433, SER 298 ,GIU 376, VAL 379, MET 450,SER 284, ASN 285	-
Ombiun	-8.5	GLY 404 ,GLU 384.	-	MET 223, PRO 407, PHE 512,	ASN 406, PHE 391, ALA 356,- SER 355	Other Pi-cation - GLU 411 Pi-

		TYR 523, ARG 522		HIS 353, VAL 518		Anion- GLU 403C-H bond HIS 410, HIS 387, HIS 513
Ascorbic acid	-5.7	ASP 121, TRP 220	-	-	TYR 213, ALA 208, ALA 207, ILE 204,ARG 124, TYR 135 ,GLU 123,ASN 211, SER 219	
trans p coumaric acid	-6.2	ARG 124, SER 517	-	ALA 207	LEU 139, TYR 135,SER 219,ASP 121 ,TYR 213, ASN 211, TRP 220, ILE 204	Other Pi-anion- GLU 123
Carbonic Acid	-3.3	HIS 353, CYS 352, SER 147, VAL 350	-	-	PHE 512, VAL 351, LEU 161, TYR 146	-
Niacin	-5.4	ASN 85, ARG 124	-	- LEU 139	LEU 81, TYR 62, ASN 136 ,LEU 140, GLU 143	-
Quercetin	-8.3	THR 301, ASP 453	-	LYS 449, LEU 375	THR 302, ASP 300, MET 299, LEU 433, SER 298 ,GIU 376, VAL 379, MET 450,SER 284, ASN 285	-

Table no: 14 Drug likeliness propertiesof isolated ligand from *coccinia grandis*

Sr. no.	Compound	Lipinski	Ghose	Veber	Egan	Muegge
1	Betasitosterol	Yes	No	Yes	No	No
2	Cucurbitacin B	Yes	No	Yes	No	Yes
3	Kaempferol	Yes	Yes	Yes	Yes	Yes
4	Thiamine	Yes	Yes	Yes	Yes	Yes
5	Ferulic Acid	Yes	Yes	Yes	Yes	No
6	Quercetin	Yes	Yes	Yes	Yes	Yes
7	Ombium	Yes	Yes	Yes	Yes	Yes
8	Ascorbic acid	Yes	No	Yes	Yes	No
9	Trans p coumaric acid	Yes	Yes	Yes	Yes	No
10	Carbonic Acid	Yes	No	Yes	Yes	No
11	Niacin	Yes	No	Yes	Yes	No

The *in-silico* analysis of chemical components of leaf extracts of*C. Grandis* was done with molecular docking disclosed the significance of drug designing for the invention of novel drugs againsts the inhibition oftargets. Most of the biomolecules from *C. grandis* leaves were shows better docking results against the active sites of

both the selected proteins 1V97 and 1UZE. Binding energy, hydrogen bond interactions and Vander Waal' s interactions for both the enzymes are listed in Tables.

3.10.3 Result of Molecular docking result examination of bioactive substances of *coccinia grandis* against the protein 1V97

β-Sitosterol shows better docking results against 1V97 and 1UZE among all other chemical components of *C. grandis* leave. β-Sitosterol is docked against1V97 with binding energy -8.9 Kcal/mol and it does not show any hydrogen bond interaction with receptor.

β-Sitosterol when docked with1UZE it exhibits -9.3 Kcal/ mol binding energy with one hydrogen bond interaction GLU 411 between the ligand molecule and receptor. β-Sitosterol's two- and three-dimensional interactions with the active region of both the receptors are depicted in figures A&B.

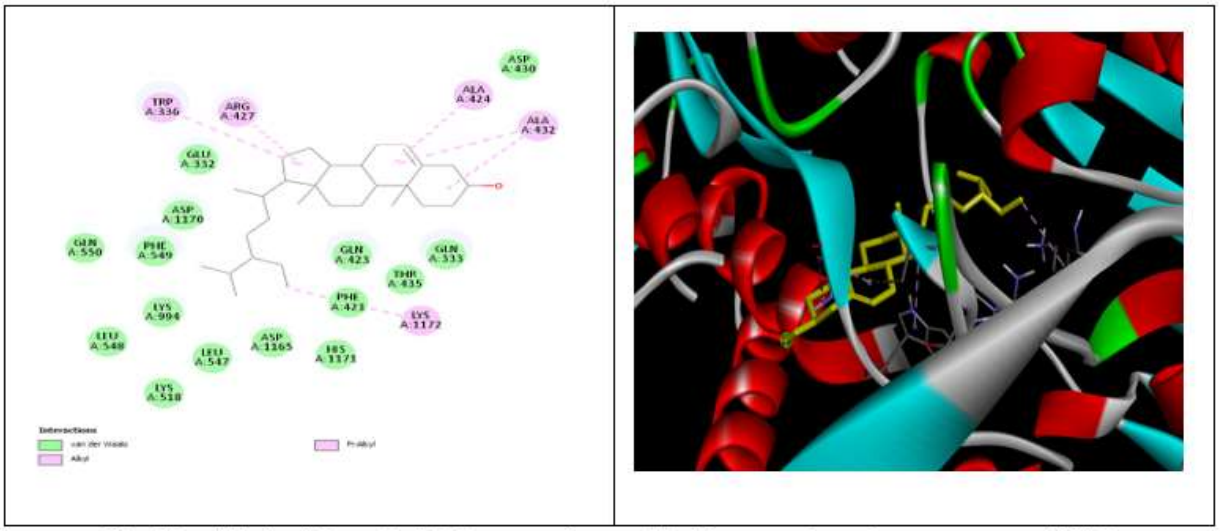


Fig. No. 13. A. 2D and 3D interactions of β-Sitosterol against receptor1V97

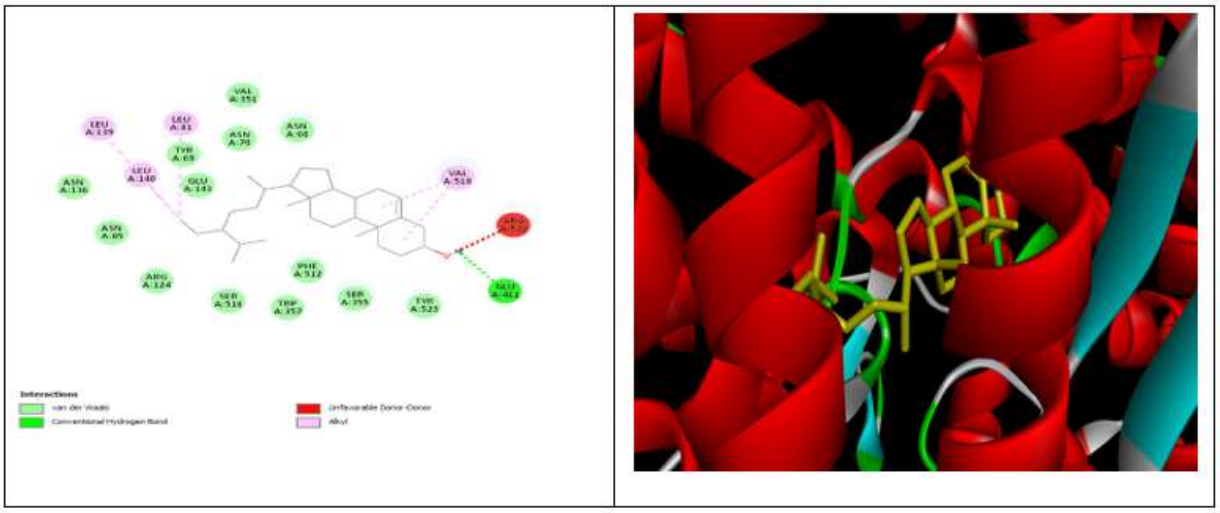


Fig. No. 14. B. 2D and 3D interactions of β-Sitosterol against receptor 1UZE

Cucurbitacin B has four hydrogen bond connections and a binding energy of -8.6 Kcal/mol with THR 354, GLY 260, GLU 263, and THR 262 when docked against 1V97. But against 1UZE, itexhibits -10.0 Kcal/mol binding energy and also has three hydrogen bond at GLU 123, ASN 211, ILE 204. Cucurbitacin B's interactions in two and three dimensions with the active site of both the receptors are depicted in figures C&D.

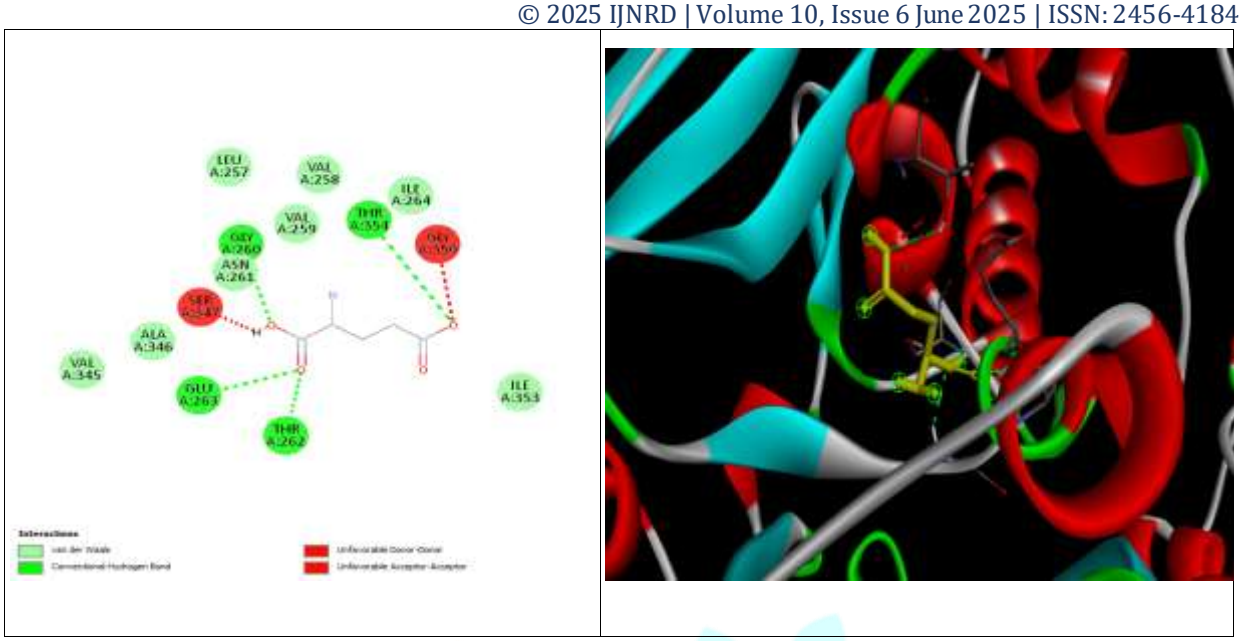


Fig. No. 15. C.2D and 3D interactions of Cucurbitacin B against receptor 1V97

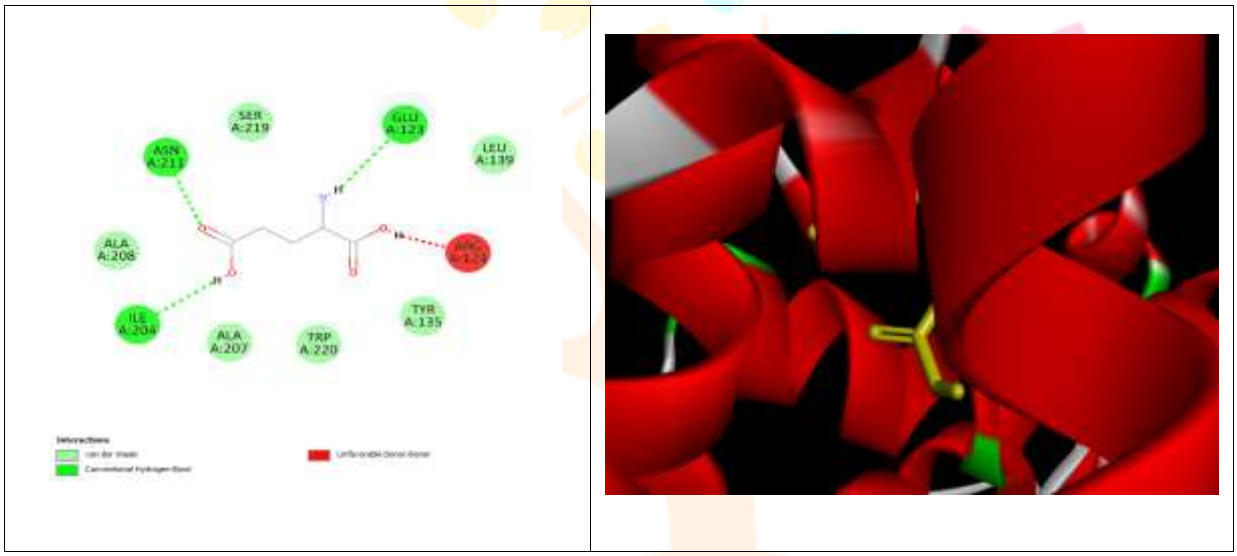


Fig. No. 16. D.2D and 3D interactions of Cucurbitacin B against receptor 1UZE

Kaempferol also has better binding energy against both the receptors 1V97and 1UZE (correspondingly, -9.1 Kcal/mol and -8.2 Kcal/mol). There are shown four hydrogen bond interactions GLU 263, SER 347, TRP 336, LYS 422 against1V97 and five hydrogen bond interactions against1UZE. Kaempferol's 2D as well as 3D interactions with the active region of both the receptors are depicted in figures E&F.

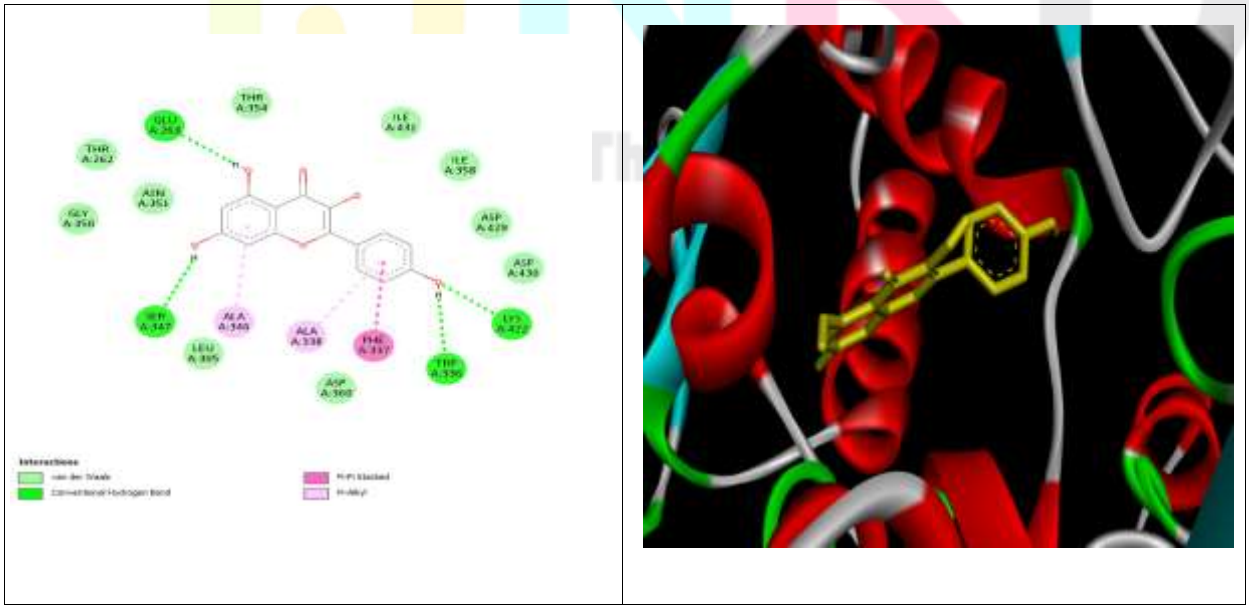


Fig. No. 17. E.2D and 3D interactions of Kaempferol against receptor 1V97

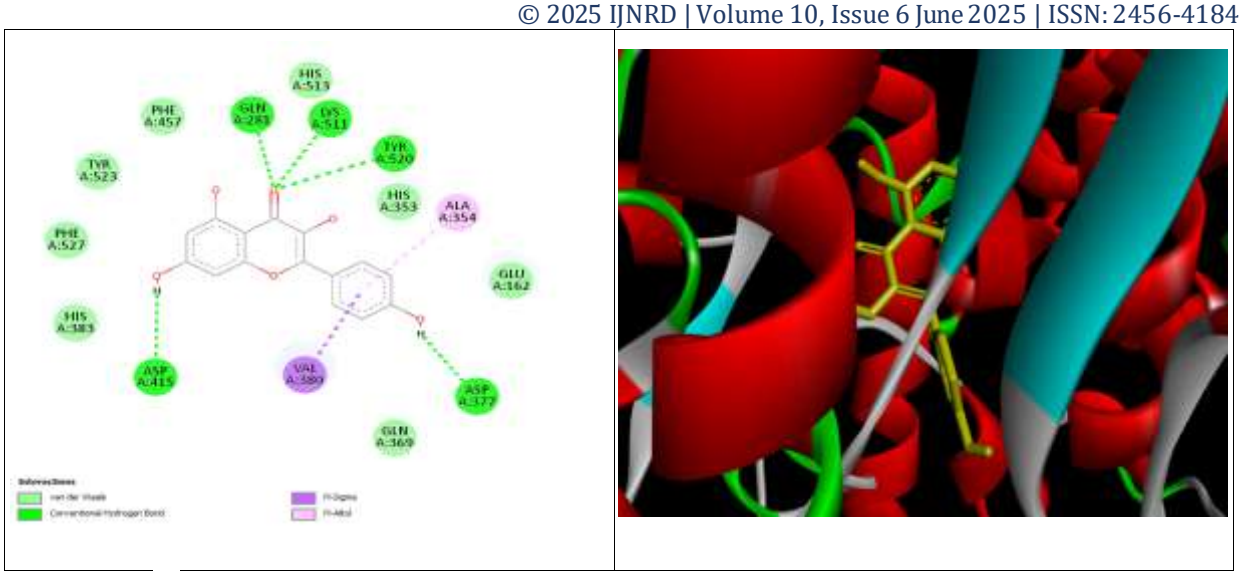


Fig. No. 18. F.2D and 3D interactions of Kaempferol against receptor 1UZE

Ferulic Acidexhibits the lowest binding energy against receptor 1V97 among the all other chemical compounds of *Coccinia grandis.* Ferulic Acid is docked against receptor 1V97 with binding energy-7.4 kcal/mol which consist of two Hydrogen bonds LEU 404, GLY 260 respectively. Against 1UZE it exhibits -6.5 Kcal/mol binding energy with two hydrogen bond interaction at ARG 124, SER 517.The 2D and 3D structure interactions are given below figure G & H.

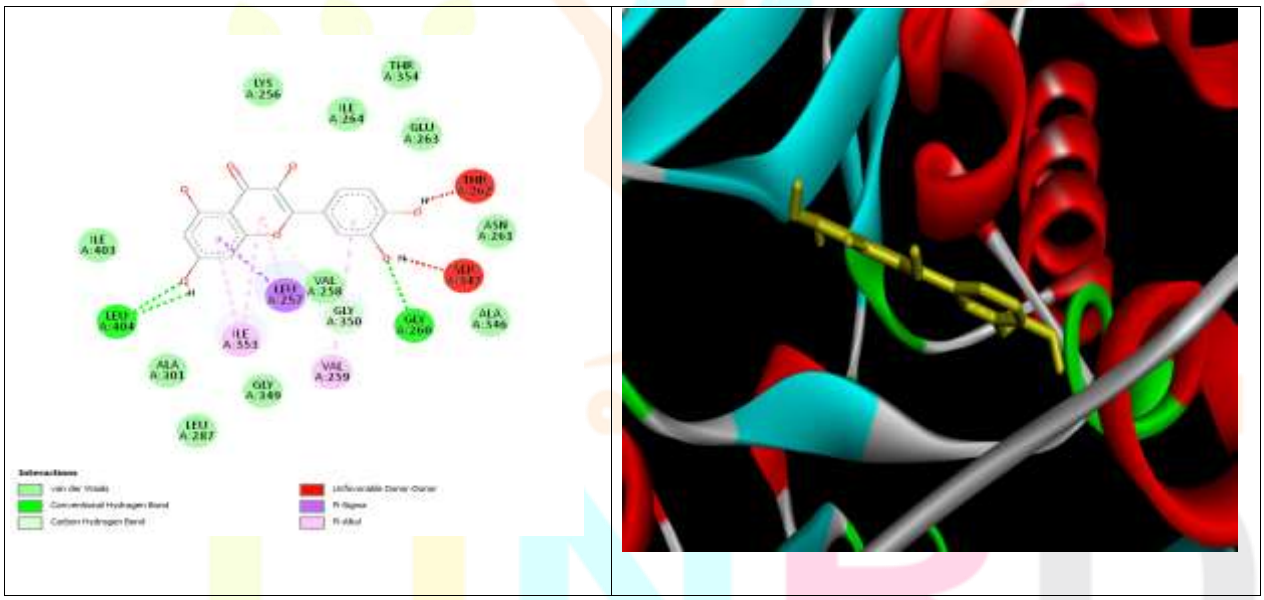


Fig. No. 19. G. 2D and 3D interactions of Ferulic Acid against receptor 1V97

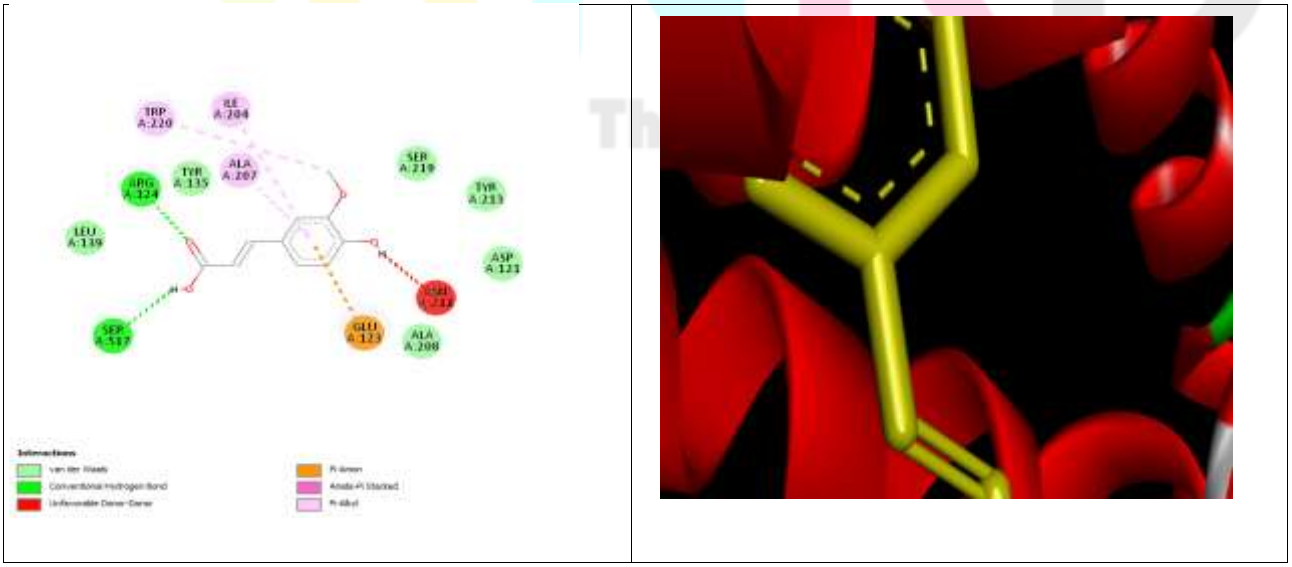


Fig. No. 20. H. 2D and 3D interactions of Ferulic Acid against receptor 1UZE

Quercet in the lowest binding energy against receptor 1V97.Quercetin is docked against receptor 1V97 with binding energy -10.1kcal/mol which consist of one Hydrogen bonds ALA 301, Against 1UZE it exhibits -8.3 Kcal/mol binding energy with two hydrogen bond interaction at THR 301, ASP 453.The 2D and 3D structure interactions are given below figure I & J.

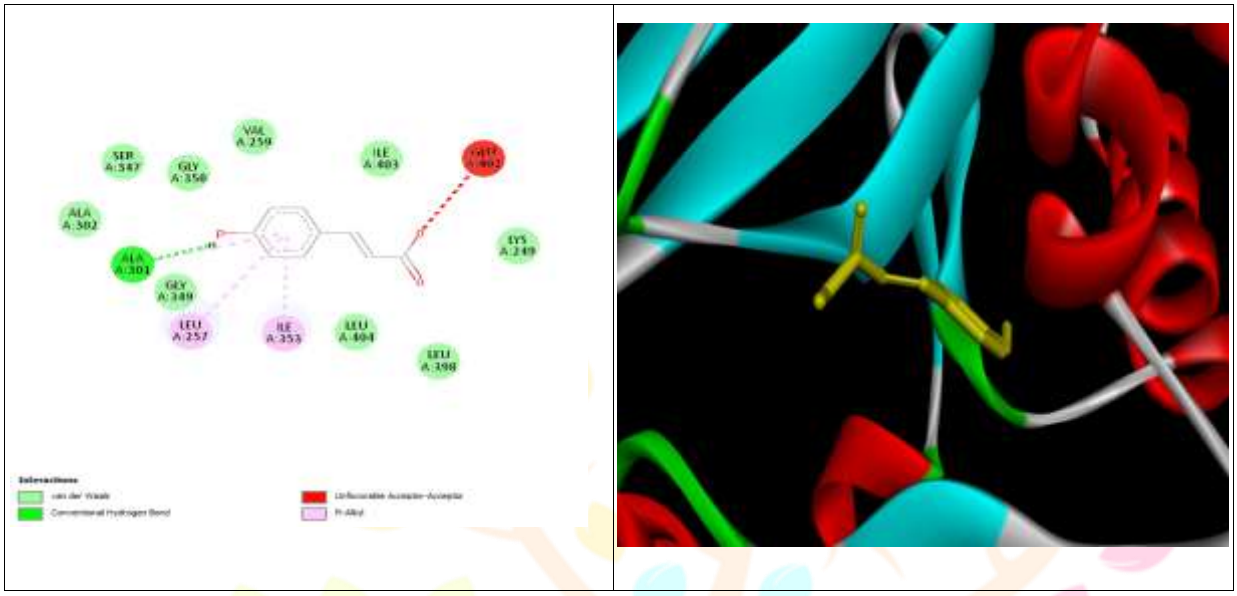


Fig. No. 21.I.2D and 3D interactions of Qurcetine against receptor 1V97

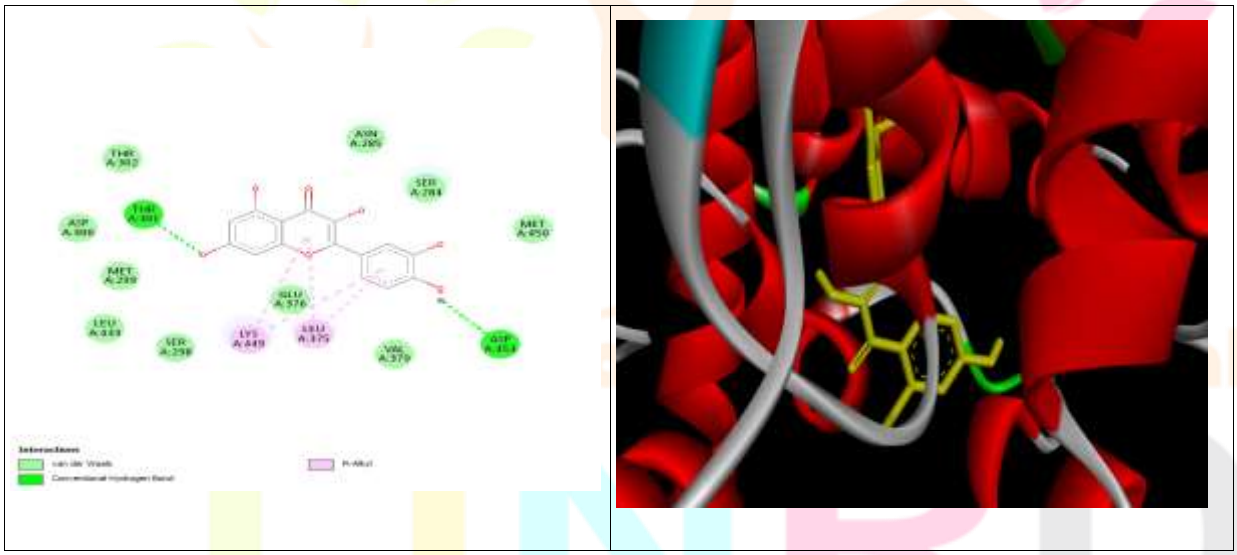


Fig. No. 22. J. 2D and 3 D interactions of Qurcetine against receptor 1UZE

Ombiun shows better docking results against 1V97 and 1UZE among all other chemical components of *C. grandis* leave. Ombiun is docked against1V97 with binding energy -8.7 Kcal/mol and it does nots how any hydrogen bond interaction with receptor.

Ombiun when docked with1UZE it exhibits Four hydrogen bonds and a binding energy of -8.5 Kcal/mol GLY 404 ,GLU 384. TYR 523, ARG 522 between the ligand molecule and receptor. The 2 Dimen. & 3Dimen.relationship of Ombiun against the active region f both the receptors are depicted in figures K&L.

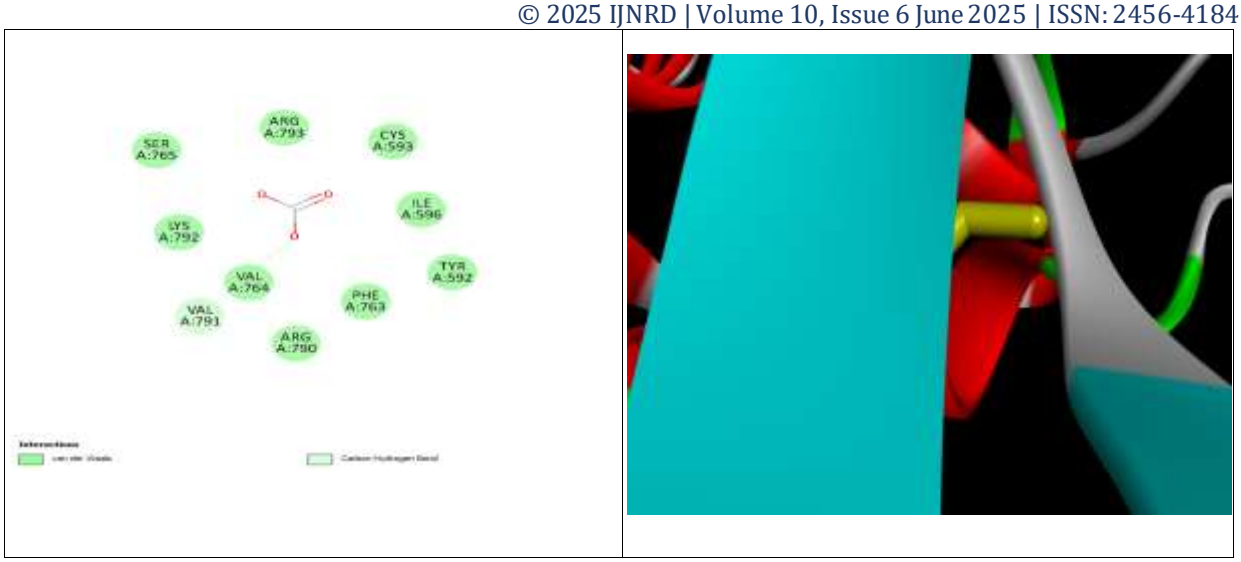


Fig. No. 23. K. 2D and 3D interactions of Ombiun against receptor 1V97

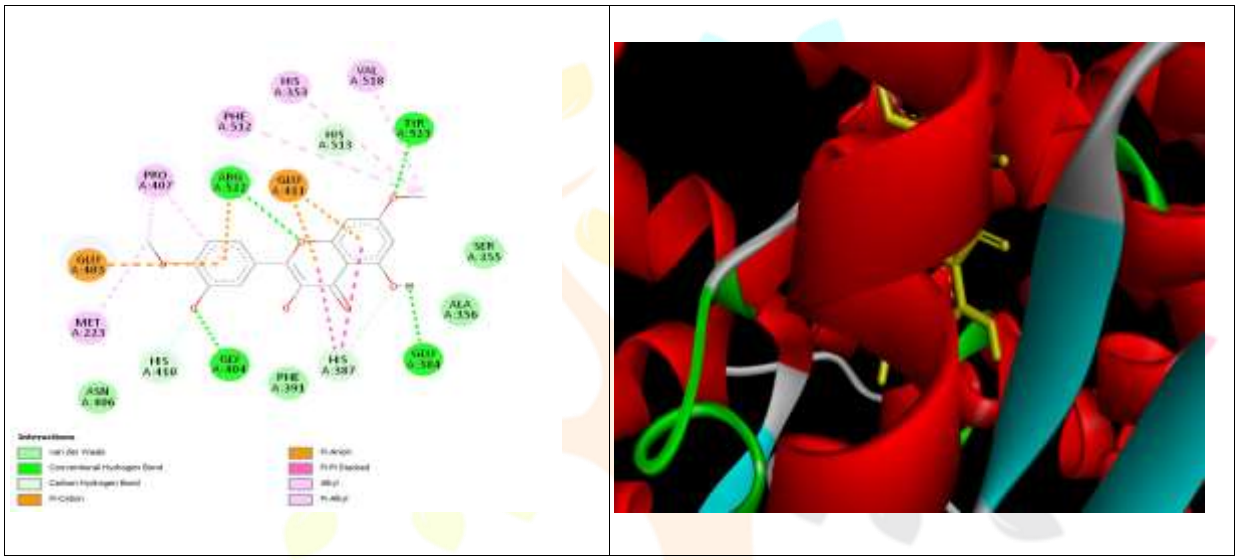


Fig. No. 24. L. 2D and 3D interactions of Ombiun against receptor 1UZE

Thiamine exhibits the lowest binding energy against receptor 1V97 and 1UZE among the all other chemical compounds of *Coccinia grandis.* Thiamineis docked against receptor 1V97 with binding energy -7.2 kcal/mol which consist of four Hydrogen bonds SER 69, SER 307, SER 306,ASN 130.Against 1UZE it exhibits -6.9 Kcal/mol binding energy with two hydrogen bond interaction at TYR 62, SER 355.The 2D and 3D structure interactions are given below figure M & N.

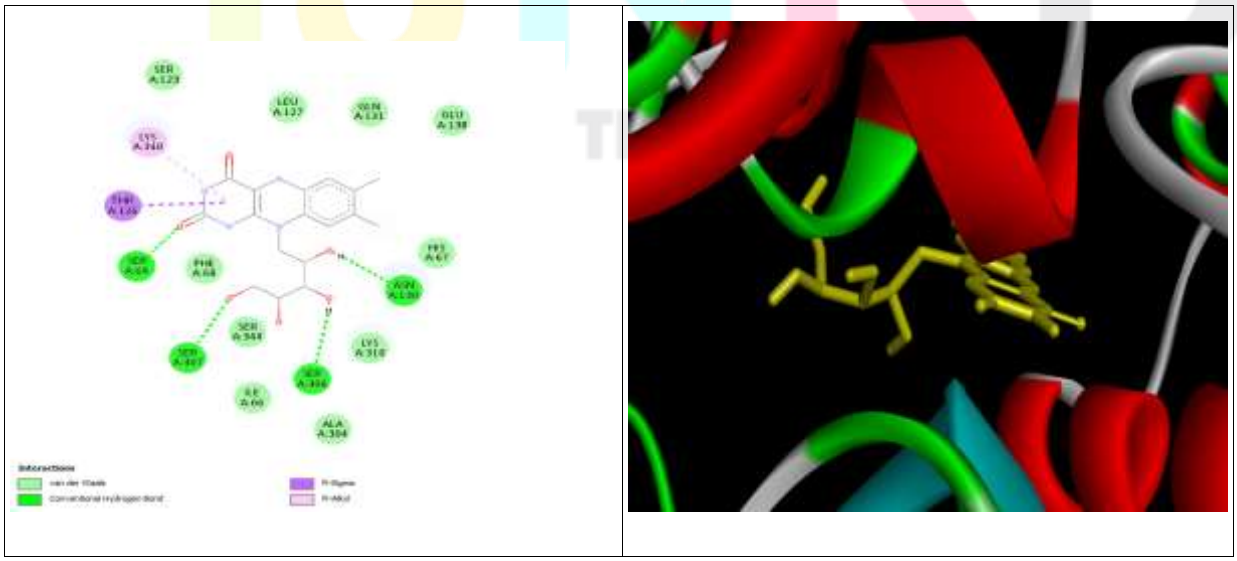


Fig. No. 25. M. 2D and 3D interactions of Thiamine against receptor 1V97

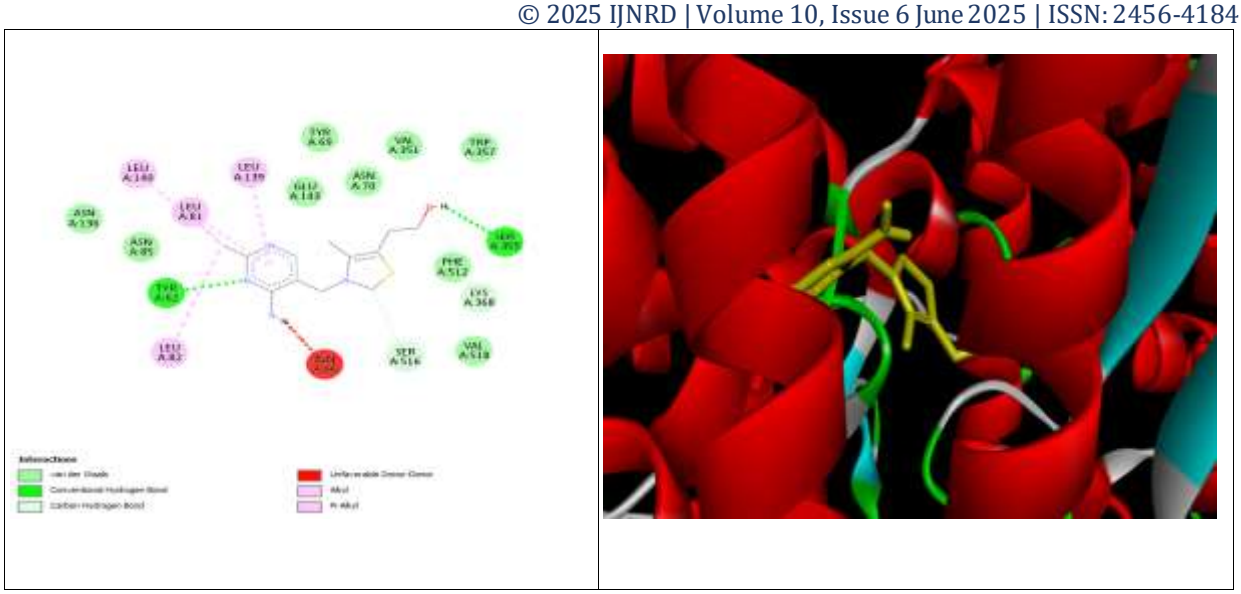


Fig. No. 26. N. 2D and 3D interactions of Thiamine against receptor 1UZE

Trans p coumaric acid exhibits the lowest binding energy against receptor 1V97 and 1UZE among the all other chemical compounds of *Coccinia grandis*. trans p coumaric acidis docked against receptor 1V97 with binding energy -6.8kcal/mol which consist of four Hydrogen bonds GLN 561, LEU 1243, LEU 1244, GLY 1183.Against 1UZE it exhibits -6.2 Kcal/mol binding energy with two hydrogen bond interaction at ARG 124, SER 517.The 2D and 3D structure interactions are given below figure O & P.

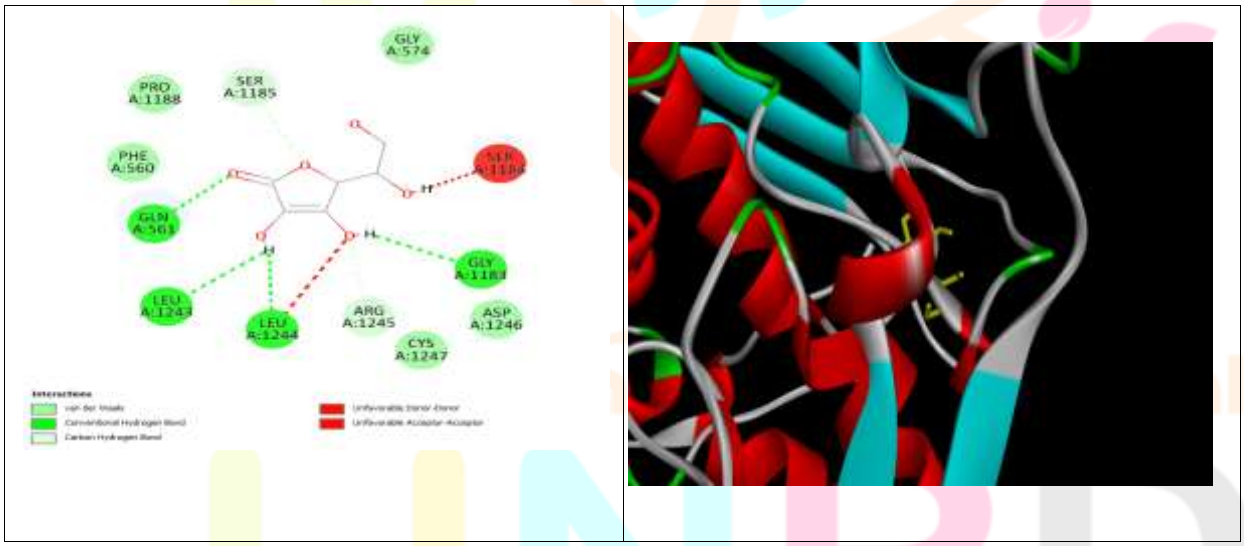


Fig. No. 27. O. 2D and 3D interactions of Trans p coumaric acid against receptor 1V97

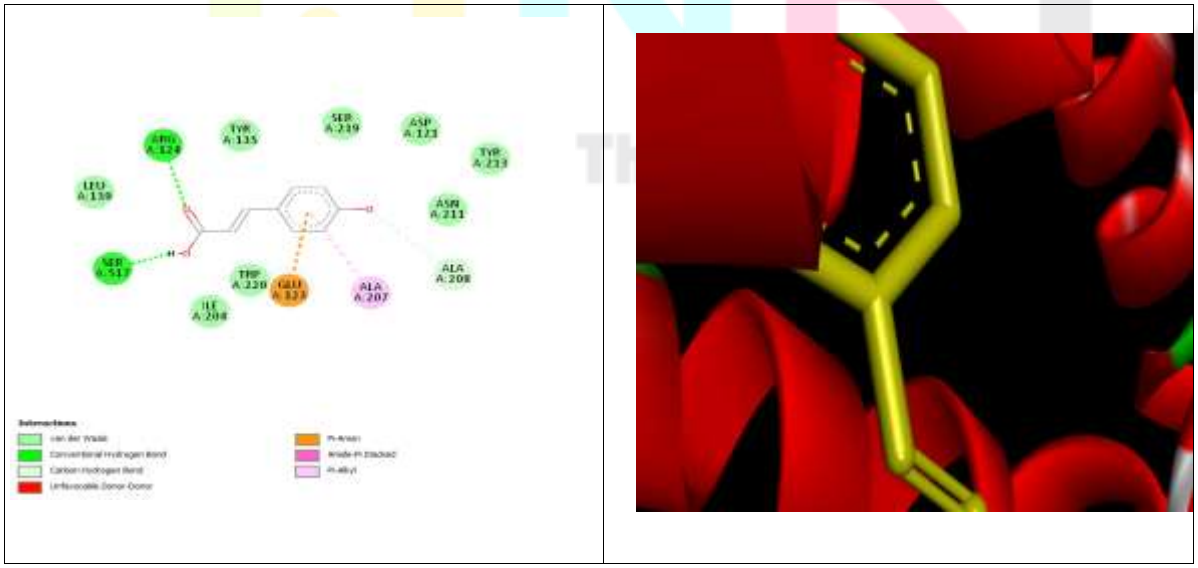


Fig. No. 28. P. 2D & 3D interactions of Trans p coumaric acid against receptor 1UZE

In-silico analysis for chemical components of leaf extracts of *C. grandis* was done Using molecular docking,

it was revealed how important drug design is for creating new medications that block targets. Most of biomolecules from *C.grandi s*leaves were shows better dockingresults against the active sites of both the selected receptor 1V97 and 1UZE. Binding energy, hydrogen bond interactions and Vander Waal's interactions for both the enzymes are listed in Tables. As the binding energy lowers, binding efficiency will increase. Similarly, as the number of As the number of hydrogen bonds between the ligand and enzyme rises, so does the binding's strength. (Kortemmne etal.,2003; Biswal etal.,2019).

4 CONCLUSION

Several polarity phytoconstituents were extracted using a soxhlet device and solvents such as nhexane, chloroform, ethyl acetate, and methanol in order to perform a phytochemical analysis on the leaf powder of Coccinea grandees. An occurrence in Saponins, phenols, flavonoids, terpenoids, glycosides, & phytosterol was demonstrated by the methanolic extract. The UV spectra of isolated extract from *Coccinia grandis*was performed using methanol. The UV spectrum showed characteristic bands of fraction in methanol atThe UV spectra of standard showed one characteristic peak at . The FTIR analysis of *Coccinia grandis* gave broad peak at 2952.75cm^{-1} which indicated the presence of phenolic O-H stretching. It showed strong peaks at 2921.35cm^{-1} which indicated the presence of alkanes whereas the peaks at1493.22and1378.31cm^{-1} attributed to existence of the aromatic ring's C–C stretching and the C–O stretch, respectively.

In-vitro anti-urolithiatic like crystal ggregation assay as compared to standard the given sample–showed moderated in vitro antiurolithiasis activity**,** nephroprotective assay at the different concentrations Sample Code **CG1** shows low percent of inhibition and against NRK 52E (Kidney Cells) cell line as equated to standard drug 5FU. On the basis of percent of viability wecan conclude that the sample CG1 may not harm the normal kidney cell lines and it may protect the kidney from the harm organ damages.

Coccinia grandis plant contains bioactive compounds like Betasitosterol, Cucurbitacin, kaempferol, Thiamine, Ferulic Acid, Quercetin, ombiun, Ascorbic acid, trans p coumaric acid, Carbonic Acid, Niacin, demonstrating ligand interactions. The Lipinsaki's method of five, or the method of five, helps identify bioactive compounds with molecular docking and drug likeliness. This helps in developing new methods for using phytomedicines in medication development. Lipinski's rule of five, which includes Log P (<+5), molecular mass (<500), 3. The quantity of donors of hydrogen bonds (<5) 4. There are less than ten hydrogen bond acceptors. 5. 40–130 molar refractivity. Molecular docking results result in the creation of novel medications against drug targets based on a structure-based drug deceitful strategy. The best binding conformation of inhibitors to enzymes was identified with the lowest energy conformation by molecular docking. Protein-ligand complexation gives plenty of pieces of information including hydrogen bonds, lipophilic interactions, π-π interactions from the protein-ligand interaction profile.

Investigation of protein 1V97 and 1UZE is done by using PDB sum software with the help of Ramchandran plot procheck analysis. According to Ramchandran plot statistics for 1V97 protein the most favoured regions is 88.7%, additional allowed region is 10.3% generally allowed region is 0.6% and disallowed region is 0.4% which are shown in fig. A. Again similarly according to Ramdrachandran plot statistics for 1UZE most favoured regions is 94.3%, additional allowed region is 5.5% generally allowed region is 0.2% and disallowed region is 0.0%.

The in-silico analysis of chemical components of leaf extracts of *C.grandis* was done with molecular docking disclosed the significance of drug designing for the invention of novel drugs against the inhibition of targets. Most of the biomolecules from *C.grandis* leaves were shows better docking results against the active sites of both the selected receptor 1V97 and 1UZE. Binding energy, hydrogen bond interactions and Vander Waal's interactions for both the enzymes are listed in Tables. As the binding energy lowers, binding efficiency will increase. Similarly, as the number of hydrogen bonds increases among the ligand and enzyme, followed by the binding's strength is also increase.

5 REFERENCES

1. Kumari P., Kumari C., Shekhar Singh P.; Phytochemical Screening of Selected Medicinal Plants for Secondary Metabolites;SSR Institute of International Journal of Life Sciences Res.;2017;*3*(4);1151-1157.
2. Shahriar M., Bahar M., Hossain I., Akhter S., Haque M. A., Bhuiyan M.A.; Preliminary phytochemical screening.: *In vitro* Anti-oxidant and cytotoxic activity of five different extracts of *Withania somnifera* root. International Journal of Pharmacy; 2012; 2: 450-453.
3. Satyavati G.V., Gupta A. K., Tandon N.; Medicinal plants of India, Indian Council of Medical Research, New Delhi, India; 1987.
4. Amir Hossain S.K., Uddin N., Salim Abu M., Haque R.; Phytochemical and Pharmacological screening of *Coccinia grandis Linn*;Journal of Scientific and Innovative Research; 2014; 3 (1): 65-71.
5. Pekamwar S. S., Kalyankar T.M., Kokate S.S.; Pharmacological Activities of *Coccinia grandis*, review; Journal of Applied Pharmaceutical Science. 2013; 3: 114-119.
6. Tamilselvan N., Thirumalai T., Elumalai E.K., Balaji R.,David E.; Pharmacognosy of *Coccinia grandis*, a review; Asian Pacific Journal of Tropical Biomedicine. 2011; 1(2): s299-s302.
7. Manimala M., Clement AtleeW., Sheik Mohammed A.; Effect of *Coccinia grandis* on ammonium chloride and ethylene glycol induced urolithiasis in rats; Int J Drug Dev. & Res., 2014, 6 (3): 138-146.
8. Varuna V., Coccinia grandis (l) voigt- review, World Journal of Pharmaceutical Research, 2018, 7 (12): 188-200.
9. Kumar M, Alok S, Jain SK, Verma A, Mahor A, Sabharwal M, Morphology, pharmacological activity, pharmaceutical preparation, doses and side effect of Coccinia indica (Wight & Arn.) &58; An overview, Journal of Coastal Life Medicine,1, 2013 ,330-336.
10. Tailwal P, A marvellous plant- coccinia indica, European Journal of Biomedical and Pharmaceutical sciences,3, 2016,232-238.
11. Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R, David E, Pharmacognosy of Coccinia grandis: a review, Asian Pacific Journal of Tropical Biomedicine,1, 2011, S299-S302.
12. Sikta SA, Mahbub J, Mou RA, Trisha KT, Dash PR, Ethnomedical profile of different parts of Coccinia cordifolia: A review, International Journal of Medical and Health Research,4, 2018, 130-136.
13. Ivy gourd (Coccinia grandis) - Technigro. Available from : www.technigro.com.au/wp-content/uploads/2016/10/24- Ivy-Gourd.pdf [Assessed on : May 2011].

14. Ivy Gourd Risk Assessment - Department of Agriculture and Fisheries. Available from: https://www.daf.qld.gov.au/- data/assets/pdf.../IPA-Ivy-Gourd-Risk-Assessment.pdf. Steve Csurhes. [First published 2008, Updated 2016].
15. Dr. Manju Maria Mathews, A Compendious Write-Up on Coccinia grandis, Betsy Sunny, Int. J. Pharm. Sci. Rev. Res.,2019, 54(2), Pages: 29-36.
16. Sargunam H, Ivy Gourd–Medicinal and nutritional values, International Journal of Current Research,9, 2017, 47604- 47607.
17. Kumar M, Alok S, Chanchal DK, Bijauliya RK, Yadav RD, Sabharwal M, An updated pharmacological activity of coccinia indica (wight & arn.), International journal of pharmaceutical sciences and research,9, 2018,456-465.
18. Harshitha Y, Prasanthi NL, Ramarao N, Coccinia grandis: a pharmaceutical review, International Journal of Pharma And Chemical Research, 4, 2018,117-124.
19. Nangare AK, Shinde AK, Pawa KK, Chavan, Review on coccinia grandis (l) voigt (ivy gourd), World Journal of Pharmaceutical Research,4,2015, 728-743.
20. Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R, David E, Pharmacognosy of Coccinia grandis: a review, Asian Pacific Journal of Tropical Biomedicine,1, 2011, S299-S302.
21. Farrukh U, Shareef H, Mahmud S, Ali SA, Rizwani GH, Antibacterial activities of Coccinia grandis L, Pakistan Journal of Botany, 40, 2008, 1259-1262.
22. Vadivu R,Krithika A, Biplab C, Dedeepya P, Shoeb N, Lakshmi KS,Evaluation of Hepatoprotective Activity of the Fruits of Coccinia grandis Linn, International Journal of Health Research,1,2008,163-168.
23. Bhaskar A, Chidamabaram R., Lakshmi Devi R., Senthamil Selvi R., Alagendran S. Protective effect of Coccinia grandis [L] against (Diethylnitrosamine) DEN induced Heptotoxicity in Wistar Albino Rats, Scholars Research Library, Der Pharmacia Lettre, 8,2016,160-165.
24. Santharam B, Divya VV, Thirupathi T,Anti ulcer activity of ethanolic, aqueous and total aqueous extracts of Coccinia grandis Linn. Voigt in pyloric ligature induced ulcers in albino rats, International journal of pharmacy and pharmaceutical sciences,5, 2013,104-106.
25. Chatterjee T.K, Umamaheswari M,Effect of fractions of Coccinia grandis on lipid peroxidation and antioxidant enzymes in oxonate induced Hyperuricaemic mice,International journal of Biomedical and Pharmaceutical sciences, 2, 2008,108-111.
26. Pavithra M.K.S., Vijayakumar L., Anjana M., Archana R., Abharna M Gayatri A., Priyadharshini G., Sivaranjani E and Sumitha C, Phytochemical and antioxidant potential of fruit and leaf extracts of coccinia grandis, International Journal of Current Advanced Research,6, 2017,3802-3805.
27. Ghosh S, Roy T, Evaluation of antidiabetic potential of methanolic extract of Coccinia indica leaves in streptozotocin induced diabetic rats, International Journal of Pharmaceutical Sciences and Research,4,2013, 4325-4328.
28. Pattanayak SP, Sunita P,In vivo antitussive activity of Coccinia grandis against irritant aerosol and sulfur dioxideinduced cough model in rodents, Bangladesh Journal of Pharmacology,4,2009,84-87.

29. Mohammed SI, Vishwakarma KS, Maheshwari VL, Evaluation of Larvicidal Activity of Essential Oil from Leaves of Coccinia grandis against Three Mosquito Species, Journal of arthropod-borne diseases, 11, 2017, 226–235.
30. Bunkrongcheap R, Inafuku M, Hirosuke OK, Hutadiloktowatana N, Wattanapiromsakul C, Sermwittayawong D, Lipid-Lowering Effects of Hexane Fraction of Ivy Gourd (Coccinia grandis L. Voigt) Root in Mice Fed a High-Fat Diet, Walailak Journal of Science and Technology (WJST),13, 2015, 815-825.
31. Deshpande SV, Patil MJ, Daswadkar SC, Suralkar U, Agarwal A, A study on anti-inflammatory activity of the leaf and stem extracts of Coccinia grandis L. Voigt, International Journal of Applied Biology and Pharmceutical technology,2, 2011,247- 250.
32. Sutar N, Garai R, Sharma UK, Sharma US, Jana GK, Singh A, Evaluation of anti-inflammatory active ity of Coccinia indica leaves extracts, Journal of Pharmacy Research,3, 2010, 2172-2173.
33. Aggarwal AS, Suralkar UR, Chaudhari SG, Deshpande SV, Garud AA, Talele SG, Analgesic and antipyretic activity of methanolic extract of Coccinia grandis L. Leaves in experimental animals, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2, 2011,175-182.
34. Bhattacharya B, Lalee A, Mal DK, Samanta A, In vivo and in vitro anticancer activity of Coccinia grandis (L.) Voigt.(Family: Cucurbitaceae) on Swiss albino mice, Journal of Pharmacy Research, 4, 2011,567-569.
35. Mohammed SI, Vishwakarma KS, Maheshwari VL, Evaluation of Larvicidal Activity of Essential Oil from Leaves of Coccinia grandis against Three Mosquito Species, Journal of arthropod-borne diseases, 11, 2017, 226–235
36. Taur DJ, Patil RY, Mast cell stabilizing, antianaphylactic and antihistaminic activity of Coccinia grandis fruits in asthma, Chinese journal of natural medicines, 9, 2011, 359-362.
37. Wagner WL, Herbst DR, Sohmer SH. Manual of the flowering plants of Hawaii (bishop museum special publication). Honolulu: University of Hawaii and Bishop Museum Press; 1999.
38. Gupta AK, Tandon N, Sharma M. Quality standard of India medicinal plants. New Delhi: Medicinal Plant Unit (ICMR); 2008, p. 157-167.
39. Suresh V Chennupati, Narayana Rao Alla*, M S C Bose, Anusha G, Sravani A, Study On Anti Urolithiasis Activity Of Some Common Medicinal Plantsalcoholic Extracts, An International Journal of Advances in Pharmaceutical Sciences, 2015, 6 (2):2784-2789.
40. Soundararajan P, Mahesh R, Ramesh T, HazeenaBegum V. Effect of Aerva Lanata on calcium oxalateurolithiasis in rats. Indian journal of experimentalbiology, 44, 2006, 981-986.
41. Van Der GK. Human Anatomy. 6th ed. New York, USA: McGraw-Hill Companies; 2001. pp. 678-83.
42. Scanlon VC, Sanders T. Essentials of Anatomy and Physiology. 5th ed. Philadelphia, USA: FA Davis Company; 2007. pp. 421-32.

43. Narayanan M., Jayashree T., and Kandasamy S.; An in vitro investigation of the antidermatophytic, antioxidant, and nephroprotective activity of *Solanum surattense*; Process Biochemistry 109; (2021):178-185.
44. Pushpa Rani V., Pranesh M., B., Shanmuga P., K.; Anitha Nancy A;Phytochemical Screening and GC-MS Analysis in Wild Variety of *Coccinia indica* –An Future Promising Therapeutic Source;American Journal of Phytomedicine and Clinical Therapeutics;2016;4(4); 098–105.
45. Bindurani R., Singh A.; Extraction, Isolation and Characterization Screening of *Coccinia grandis*; Journal of Drug Delivery & Therapeutics. 2019; 9(3):238-245.
46. Mohd A., Moh T., Khan A., and Mansoor A.; Biocidal and Antinemic Properties of Aqueous Extracts of *Ageratum* and *Coccinia* Against Root-Knot Nematode, Meloidogyne Incognita In Vitro;The Journal of Agricultural Sciences; 2017;12; 108-122.
47. Jha R., Tahil Raman P., Patel D.; Phytochemical analysis and in vitro urolithiatic Activity of *Peltophorum pterocarpum* leaves (DC) Baker; Journal of Medicinal Plants Studies; 2016; 4(3): 18-22.
48. Mamillapalli V., Khantamneni P.; Phytochemical &*In-Vitro* Antiurolithiatic Studies on The Leaf Extracts of *Bauhinia varigata Linn*; International Journal of Pharmaceutical Sciences and Research; 2016; 7(10): 4074-4084.
49. Reshma.,Aruna P., Brindha P.; *In Vitro* Anti-Inflammatory, Antioxidant And Nephroprotective studies on leaves of *Aegle marmelos* and *Ocimum sanctum*;Asian Journal of Pharmaceutical and Clinical Research;2014; Vol.7(4); 121-129.
50. Kumar M., Shashi A., Kumar J. S., Verma A.; *In –Vivo* study *of* Antilithiatic Activityon the fruits of *coccinia indica (Wight and Arn.)* Ethelyne glycol Induce Lithiatic in Rats; International Journal of Pharmacognosy; 2014; 1(1): 51-58.
51. Syed Zeenat S., Krishna B., Kandukuri V.;Antimicrobial Activity of the fruit extracts of *Coccinia indica*;African Journal of Biotechnology; 2009; 8 (24); 7073-7076.
52. Chatterjee T., Umamaheswari M.; *In Vitro* Antioxidant Activities of The Fractions of *Cocciniagrandis* L. Leaf Extract; Afr. J. Trad. CAM (000) 5 (1): 61 – 73.
53. A.Sivaraj, B. Preethi Jenifa, M. Kavitha, P. Inbasekar et al Antibacterial activity of Coccinia grandis leaf extract on selective bacterial strains; Journal of Applied Pharmaceutical Science ; 2011:1(7): 120-123.
54. M. A. A. K.Munasinghe, C. Abeysena, I. S. Yaddehige, T. Vidanapathirana, Blood Sugar Lowering Effect of Coccinia grandis (L.) J. Voigt: Path for a New Drug for DiabetesMellitus Experimental Diabetes Research ; 2011;3;201-201.
55. S.V Deshpande1, M. J. Patil2, S.C. Daswadkar1, U. Suralkar3, A. Agarwal3 A study on anti-inflammatory activity of the leaf and stem Extracts of coccinia grandis . Voigt International Journal of Applied Biology and Pharmaceutical Technology; 2011; 2(3): 211-214.
56. "Wealth of India", A dictionary of Indian raw materials and industrial products. Raw material, New Delhi; 1992; 4; 312.

57. S.P. Dhanabal, C.K. Koate, M. Ramanathan, K.Elango et al The hypoglycemic activity of Coccinia grandis Wight & Arn. And its influence on certain biochemical parameters. Indian J pharmacol: 2004; 36: 244-250.
58. Rahuman A A & Venkatesan Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species; J of Parasitol Res : 2008: 103 : 133-139.
59. Pacific island ecosystems at risk (2003). "invasive plant species: Coccinia grandis". retrieved 10 February 2010.
60. NMC Crees (1997). "Scarlet Gourd in Saipan". archived from the original on 11 January 2006. retrieved 10 February 2010.
61. Suresh Babu S K. V., Rajan. A promising triploid of little guard. Journal of Tropical Agriculture. 2001; 39 : 162-163.
62. Ajmal Ali M. and Pandey Arun K. Systematic Studies on the Family Cucurbitaceae of Eastern Bihar, India. Cucurbit Genetics Cooperative Report. 2005-2006;28-29-66-69.
63. Deokate U. A. and Khadabadi S. S.. Pharmacology and photochemistry of Coccinia indica. Journal of Pharmacognosy and Phytotherapy. 2011; 3(11): 155-159.
64. Bhattacharya Bolay et al., In vitro evaluation of antifungal and Antibacterial activities of the plant Coccinia grandis (L.) Voigt. (FamilyCucurbitaceae). Journal of Phytology. 2010; 2(11): 52-57.
65. Bulbul Israt Jahan., Nathan Laizuman., Haque Mahmuda. Antibacterial Cytotoxic and antioxidant activity of chloroform, n-hexane and ethyl acetate extract of plant Coccinia cordifolia. Agriculture and Biology journal of north America. 2011; 2(4): 713-719.
66. Tamilselvan N et al.,. Pharmacognosy of Coccinia grandis: a review. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(1): S299- S302.
67. Deshpande S. V., et al . In vitro antioxidant study of petroleum ether chloroform and ethyl acetate fractions of coccinia grandis stems extract. International journal of chemical sciences. 2011; 9 (1) : 80-86.
68. Girish C., et al. Evaluation of Antiulcer Activity of Coccinia grandis Leaves. Research Journal of Pharmacology and Pharmacodynamics. 2011; 3: 2011.
69. Sumaryono W, Proksch P, Wray V, Witte L L Qualitative and Quantitative analysis of the phenolic constituents from Orthosiphon aristatus. Planta medica : 1991:57:176- 180
70. Douglar Skoog A., James Holler., Fer Nieman., and Timothy, A “Principles of Instrumental Analysis”, London, Thomson Brook, 2005; pp. 350-432.
71. Willard, “Instrumental Methods of Analysis”, New York: D. Van Nostrand company (cannada) Ltd, 4th ed,1965 pp. 180-445.
72. Silverstein., “Spectrometric Identification of Organic Compounds”, New York, John willey & sons, 6th ed., 1998;pp. 1-48.
73. 5. Hennquin C, Lalanne V, Lacour B, Drueke T. A new approach to studying inhibitors of calcium oxalate crystal growth. Urol Res. 1993; 21(2): 101-8.

74. Prachi K, Jaya S, Arun K. In-vitro evalution of Coleus aromaticus leaves for antilithatic activity. Int J pharmacol. 2014; 1(1): 45-50.
75. Patel PK, Patel MA, Vyas BA, Shah DR. Antiurolithiatic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) against ethylene glycol induced urolithiasis in rats. J Ethnopharmacol. 2012; 144(1): 160-70.
76. Liochev SI, Benov L, Touati D, Fridovich I. Induction of the soxRS regulon of Escherichia coli by superoxide. J Biol Chem. 1999; 274(14):9479–81.
77. Udo EE, Jacob LE, Mathew B. A cadmium resistance plasmid, pXU5, in Staphylococcus aureus, strain ATCC25923. FEMS Microbiol Lett. 2000; 189(1):79–80.
78. Wang HW, Wang FQ, Tao XY, Cheng HR. Ammonia-containing dimethyl sulfoxide: An improved solvent for the dissolution of formazan crystals in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Analytical Biochemistry. 2012; 421(1):324–6.
79. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023 Aug 17;13(1):13398.
80. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011 Jun;7(2):146-57.
81. Nandini Asha R, Ravindran Durai Nayagam B, Bhuvanesh N. Synthesis, molecular docking, and in silico ADMET studies of 4-benzyl-1-(2,4,6-trimethyl-benzyl)-piperidine: Potential Inhibitor of SARS-CoV2. Bioorg Chem. 2021 Jul;112:104967.
82. El fadili M, Er-Rajy M, Kara M, Assouguem A, Belhassan A, Alotaibi A, Mrabti NN, Fidan H, Ullah R, Ercisli S, et al. QSAR, ADMET In Silico Pharmacokinetics, Molecular Docking and Molecular Dynamics Studies of Novel Bicyclo (Aryl Methyl) Benzamides as Potent GlyT1 Inhibitors for the Treatment of Schizophrenia. *Pharmaceuticals*. 2022; 15(6):670.

International Research Journal
IJNRD
Research Through Innovation