International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

In-Silico Molecular Docking Analysis of Phytochemical Profiling of kokum (Garcinia indica), An overlooked fruit of India

Ranjan Singh[1], Sumanth Kumar Sella[2], Naveen Kumar S[3], Sneha Asthana[4], Ajijur Rehman[5]

[1,2,4]Student, NIMS University Rajasthan,Jaipur -303121
[3]Student, Pondicherry University, Puducherry, 605014, India
[5]Assistant Professor, NIMS University Rajasthan,Jaipur -303121

Abstract:
In various regions worldwide, especially in South Asia, the traditional use of the Kokum plant, scientifically known as Garcinia indica, has been prevalent for a long time. Kokum is recognized for its unique phytochemical composition, which includes anthocyanins, hydroxycitric acid, xanthochymol, ascorbic acid, and garcinol. Due to its antibacterial, antioxidant, and anti-inflammatory properties, Kokum is considered a valuable option for numerous medical purposes. The traditional use of kokum suggests that it has been an effective remedy for various ailments, and further research on microbiological targets and their potential applications in modern medicine or food preservation. Asthma is a substantial global health problem with increasing prevalence rates in many countries. Asthma prevalence in India had previously been estimated to be around 3% (30 million patients out of 300 million), with prevalence rates between 4% and 20% in children and 2.4% in individuals over the age of 15. It is expected to rise in the upcoming decades and is one of the main causes of illness and death in rural India. The aim of this study was to identify putative bioactive chemicals present in the kokum fruit and investigate their interactions with the Asthma proteins by molecular docking modelling. Anthocyanin had a docking score of -8.27 kcal/mol for 3rze, -8.28 kcal/mol for 7f61, and -6.31 kcal/mol for 7fy6. The consequence of Lipinski rule recommends that anthocyanin is the best curative drug for asthma. Docking results verify the application of anthocyanin as a potential and natural therapeutic agent to treat diseases. Further laboratory tests are necessary to confirm these experimental results, though.

Keywords: Kokum, antioxidant, anti- inflammatory, Antihistamine, asthma, Garcinia indica, Molecular Docking, Lipinski’s Rule, Swiss ADME.

1. Introduction

Native fruits are an important source of nutrition for the local community, especially in remote rural locations where food is either less available or more expensive, particularly for those in need. Throughout human history, all societies have utilized medicinal herbs as a means of treatment, making it the most ancient medical practice. India's native kokum tree (Garcinia indica), a member of the Guttiferae family, mostly grown in the Western Ghats of India, specifically in the states of Kerala, Karnataka, Maharashtra, and Goa. The tree produces fruit every year in the summer, from March to May. It is sometimes referred to as an Indian spice and is a popular culinary ingredient because of its enjoyable, acceptable flavor and sweet, acidic taste.

Kokum is a tropical evergreen tree related to mangosteens. It is a thin tree with sloping branches that can reach heights of up to 15 meters (50 feet). The leaves are half oblong, and the thin bark is lined. The fruit has 5-8 seeds and is spherical in form, dark purple in hue, and about 4 cm (1 in) in diameter. Kokum is collected from its natural habitats, planted in indoor gardens, and produced in small quantities as a crop that is fed by rain. The kokum tree is a slim but incredibly resilient evergreen that doesn't require complex watering or the application of chemical-based pesticides, herbicides, or fertilizers. Kokum trees typically grow in swamps, woodlands, and riverbanks. Additionally, plantlets can be produced through the process of tissue culture and fortuitous bud development on mature seeds. The ripe kokum fruit has a deep purple or red hue with hints of yellow. It has three to eight large seeds embedded in a white pulpy substance, resembling orange segments in a regular pattern, within a red acid pulp.

Figure 1: (a) KOKUM FRUIT, (b) KOKUM SEED (c) DRIED KOKUM

Every portion of Garcinia indica, including the fruits, rind, seeds, and other sections, has been widely used for a variety of industrial, medicinal, and culinary purposes. It has long been an ingredient in many Indian recipes as an acidulant. This crop is becoming more and more significant due to the wide range of applications for its fruits, which include high-quality beverages and therapeutic uses. The fruit kokum is packed with many bioactive components that have anti-fungal, antibacterial, and antioxidant qualities. Its effectiveness against multiple cancer cell lines, including those from the liver, breast, and leukemia, has been shown by scientific studies. Additionally, kokum has antihistamine and anti-inflammatory qualities. Kokum has been used traditionally for a long time as a drug for the treatment of skin infections, diarrhea, and wound rehabilitation.

2. Chemical Composition of Kokum

Many chemical substances are present in Garcinia indica, which is easily accessible in India. Among these are derivatives of polyisoprenylated benzophenones, like Iso garcinol and garcinol. Six significant chemical components are present in kokum rind: hydroxycitric acid, anthocyanin pigment, garcinol, iso-garcinol, xanthochymol, and ascorbic acid. Garcinol is a yellow pigment that dissolves in fat. Hydroxycitric acid is a physiologically active chemical that is used as an acidulant and has been demonstrated to drastically lower body weight. The Kokum fruit's pH ranges from 1.5 to 2.0, which naturally gives it more acidity. Additionally, it has 2.4% pigment, which is a 4:1 blend of two anthocyanins: cyanidin-3-sambubioside and cyanidin-3-glucoside. Kokum contains many chemical constituents, as shown the T**able: 1**

S. No	CHARACTER	VALUE
1	Moisture (%)	80
2	Protein (N x 6.25) %	1.92
3	Crude Fat (%)	10
4	Crude fiber (%)	14.28
5	Total Ash	2.57
6	Carbohydrates by Difference (%)	35
7	Starch (%)	1
8	Pigment (%)	2.4
9	Tannin (%)	2.85
10	Pectin (%)	5.71
11	Ascorbic Acid (%)	0.06
12	Acid (as hydroxyl citric acid)	22.8

Table 1: CHEMICAL CONSTITUENTS OF KOKUM

3. Review of Literature

3.1 Kokum's nutraceutical qualities

Three main chemical compounds found in kokum are anthocyanin, hydroxycitric acid, and garcinol; each of these has nutraceuticals qualities. These substances are all found in kokum rinds.

3.1.1 Garcinol -

Garcinol is a polyisoprenylated benzophenone derivative that inhibits p300 and PCAF histone acetyltransferases. Garcinol, a yellow fat-soluble pigment, is present in the rinds of Kokum in a concentration of 2–3%. This makes it a potent antioxidant, and it is also known as camboginol, a triisoprenylated chalcone. The β-diketone moiety makes it like curcumin, a well-known antioxidant. The β-diketone moiety makes it like curcumin, a well-known antioxidant. Garcinol's molecular weight is 602.80 and its chemical formula is $C_{38}H_{50}O_6$, and its melting point is 122°C. It is crystallizing from the hexane extract of the fruit rind. Garcinol has been looked up for its anti-cancer, anti-ulcer, antioxidant, and antiglycation potential.

Hydroperoxy derivatives of cambogin and garcinol, also known as Iso garcinol, can be formed when garcinol scavenges alkyl-peroxyl radicals. Like garcinol, Iso garcinol has comparable biological effects and possesses strong antioxidant properties. These substances can cause human leukemia HL-60 cells to undergo apoptosis; they can also prevent the production of NO radicals and the expression of the iNOS gene in response to LPS. Consequently, research has demonstrated that garcinol exhibits superior anticancer efficacy compared to curcumin. Potent growth-inhibitory effects of garcinol are shown in all intestinal cells, although they are more effective against cancerous cells than in normal ones. Garcinol can therefore be used, at specific concentrations, to stop the proliferation of cancer cells.

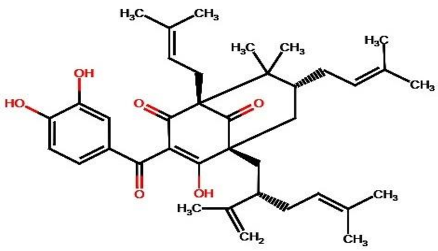


FIGURE 2: - STRUCTURE OF GARCINOL

3.1.2 Anthocyanins -

The two major anthocyanin pigments found in Kokum are characterized as cyanidin-3-glucoside and cyanidin-3-sambubioside. Thin-layer chromatography, HPLC, mass, and NMR spectroscopy have all been used to identify them. The percentage of anthocyanins consists in all fruit biomass is about 2.4%. Anthocyanins are produced through the phenylpropanoid pathway and are members of the parent family of chemicals known as flavonoids. All the tissues of higher plants, such as the leaves, stems, roots, flowers, and fruits, are susceptible to them. Anthocyanins are created by combining anthocyanidins with sugars. Many distinct color compounds are generated by chemically combining anthocyanidin pigment's basic structure with glycosides and acyl groups. The most prevalent acyl groups in nature are coumaric, caffeic, ferulic, p-hydroxy benzoic, synapic, malonic, acetic, succinic, malic, and oxalic, whereas the most prevalent sugar groups in nature are glucose, rhamnose, xylose, galactose, arabinose, and fructose. It has been demonstrated that anthocyanins have potent antioxidant properties. Anthocyanins can block oxidative enzymes, scavenge free radicals, prevent the oxidation of ascorbic acid, and lower the risk of cardiovascular disease and cancer.

Anthocyanins also affect lipid peroxidation. They are better anti-lipid peroxidation agents than α-tocopherol. Together with anthocyanins like cyanidin-3-glucoside, bioflavonoids like leucoanthocyanidins, catechins, and flavanols have demonstrated effects to strengthen and increase capillary permeability, speed up the metabolism of ethanol, and lessen eremitic responses and inflammations.

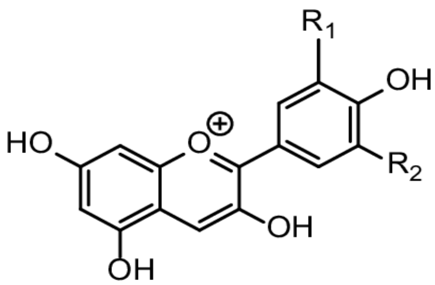


FIGURE 3: - STRUCTURE OF ANTHOCYANIN

3.1.3 Hydroxycitric acid -

Kokum contains hydroxycitric acid (HCA), one of the main acids. HCA can also be found in other species of Garcinia, including G. cambogia and G. atroviridis. Kokum contains 23% of HCA on dry basis. The rind of the fruit of Garcinia indica contains hydroxycitric acid in both free acid and lactone forms. HCA is also called Garcinia acid and can be extracted from rinds using both thermal and non-thermal techniques. Pure HCA crystals can be obtained by using acetone. Another thermal approach for separating HCA involves extracting it with deionized water and then condensing it using an osmotic membrane distillation technique using a hydrophobic polypropylene membrane. Additionally, bacteria like Bacillus megaterium G45C and Streptomyces sp. U121 have been shown to produce HCA.

HCA-containing extract has demonstrated its ability to effectively lower the body's synthesis of fat by 40–70%. It gently and gradually burns fat without activating the central nervous system. Today's consumer battles daily to prevent obesity, which is typically caused by stress, inadequate diets, eating too much, and inactivity. When we eat a meal high in carbohydrates, glucose is used up partially and stored in the muscles and liver as glycogen. Weight gain results from the extra glucose being transformed into lipids and deposited as fat throughout the body.

Hydroxyl groups are present at the second and third carbon atoms in HCA. HCA contains two sets of Dia stereoisomers since it possesses two asymmetric carbons. The free form of HCA is not stable and is commercially available as a calcium salt. Moreover, potassium hydroxy citrate is produced when HCA is treated with KOH.

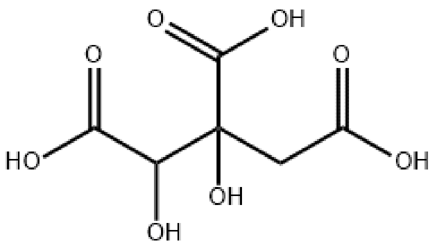


FIGURE 4: - Hydroxycitric acid

4. PHARMACOLOGICAL PROPERTIES OF KOKUM (Garcinia indica) FRUIT

In Ayurveda, kokum (Garcinia indica) is an herb with medicinal properties that is used to cure a variety of health issues, including cancer, coronary artery disease, diarrhea, and liver damage. Kokum exhibits various phytochemical features like anti-ulcerogenic, cardio-protective, anticancer, chemo-preventive, radical scavenging, and anti- obesity effects. Additionally, it helps with bladder pain, diarrhea, acne, and cardiac issues. Both the cosmetics and non-confectionery industries have strong demand for kokum butter.

4.1 ANTIOXIDANT ACTIVITY

Plants that contain flavonoids or phenolic molecules are known to possess antioxidant qualities. Because of its strong antioxidant content, kokum has special health benefits. Overproduction of free radical's damage lipids, protein, DNA, and other macromolecules. These radicals include reactive oxygen species (ROS) and reactive nitrogen species (RNS). Thus, the application of antioxidants is indispensable to obstruct the formation and offset the adverse influence of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The presence of different natural compounds in kokum could offer antioxidant benefits. The components consist of citric acid, malic acid, polyphenols, carbohydrates, anthocyanin flavonoids, and ascorbic acid. Kokum contains a significant number of anthocyanins, with the main types being cyanidin-3-glucoside and cyanidin-3-sambubioside. The rat model of oxidative stress was utilized to assess the antioxidant properties of water-based extracts derived from the fruit rind of G. indica. The extracts were subjected to testing to ascertain their potential in inhibiting lipid peroxidation and shielding against oxidative stress induced by chronic ethanol consumption in rats. The findings indicate that the extracts effectively decreased lipid peroxidation and replenished the levels of key antioxidant enzymes including SOD, CAT, GPx, and GR. The results indicate that the fruit rind of G. indica possesses strong antioxidant properties and may help prevent oxidative harm in the liver.

4.2 ANTI-OBESITY

Kokum has been traditionally employed in the Ayurvedic system of medicine to tackle health issues related to obesity. Various research investigations have demonstrated that hydroxycitric acid (HCA), a substance present in kokum, displays characteristics that combat obesity. The intake of HCA has been found to decrease appetite, inhibit fat synthesis, reduce food consumption, and ultimately lead to weight loss. The study highlights the potential of kokum and HCA as beneficial resources in addressing problems associated with obesity.

Extensive research has been conducted to explore the potential anti-obesity effects of G. indica extract (GIE) obtained from fruits. Research on the process of adipogenesis in fibroblast cell line 3T3L1 cells has been carried out in vitro, and the effects of anti-obesity diet on C57/BL6 mice have been studied. Extensive research has been carried out to investigate adipogenesis in fibroblast cell line 3T3L1 cells using in vitro methods. Moreover, the impact of these cells on combating obesity has been examined in C57/BL6 mice that were fed a high-fat diet. G. indica fruit berries contain a high concentration of polyphenols that are effective in reducing oxidative stress and problems associated with obesity. Moreover, the research has shown the fruit's potential for growth as a functional and nutraceutical food.

4.3 ANTI-DEPRESSANT

The high prevalence of depression and anxiety results in a significant impact on psychosocial well-being. Although pharmacotherapy is often the go-to treatment option, it presents several challenges such as sedation, amnesia, tolerance, psychomotor effects, and dependence. Consequently, new psychopharmacological drugs are necessary to provide immediate effects and minimize side effects. In addition, G. indica has been proven to possess significant antidepressant activity in different animal models. The potential of this substance as an antidepressant has been demonstrated by its ability to decrease the duration of immobility in forced swim and tail suspension tests. The extract(s) derived from the rind of G. indica fruit have demonstrated the ability to hinder the activity of monoamine oxidase (M A O), resulting in elevated levels of neurotransmitters such as serotonin and norepinephrine. These increased levels of neurotransmitters have been associated with positive effects on depression. Furthermore, G. indica has demonstrated the ability to counteract hypothermia caused by reserpine, a widely utilized model for evaluating potential antidepressant substances.

4.4 Anti-cancer Activity

Findings from studies have revealed that garcinol, Iso garcinol, and xanthochymol, the predominant phytochemical components discovered in Kokum, exert an influence on the growth and propagation of different human leukemia cell lines. These substances cause cancer cells to undergo apoptosis by activating caspase 3. It was shown that xanthochymol and Iso garcinol had more growth-inhibiting properties than garcinol.

Garcinol, a prominent plant compound derived from G. indica fruits, has been linked to the newly uncovered cancer-fighting properties of G. indica. Garcinol plays a key role in stalling the growth of tumor cells by inhibiting transcription factors NF-κB and JAK/STAT3.Garcinol has shown promising results in vitro against various cancer cell lines, including those found in the colon, breast, prostate, head and neck, and hepatocellular. Additionally, studies have shown that garcinol has the ability to trigger apoptosis in pancreatic cancer cells, ultimately leading to cell death. The outcomes point towards the potential use of G. indica and garcinol as a potential anticancer agent in the future.

4.5 Anti-Aging Activity - The natural aging process is often marked by the onset of wrinkles and skin that begins to sag. The reduction in skin elasticity, caused by the activity of elastase, is a significant factor leading to sagging skin over time. Aging also leads to a decline in hyaluronic acid levels, which in turn causes the skin to become dry and develop wrinkles. Consequently, it is necessary to conserve the matrix metallic protein by inhibiting the function of matrix metalloproteinases. Certain plant pieces are known to possess strong antioxidant properties. The fruit containers of Garcinia indica have been found to contain Garcinol and cambogia, which are known for their antioxidant properties attributed to the presence of a phenolic group. Research has shown that indica plants speed up the aging process. Researchers separated the methanolic extract (ME) into two fractions—a water fraction (WF) and a raw ethyl acetate fraction—in order to conduct more study. Testing was then done on these fractions to see how well they inhibited the activities of hyaluronidase and elastase.

4.6 Neuro-protective activity - Neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and Huntington's disease are characterized by the abnormal production of reactive oxygen species (ROS) and the accumulation of certain proteins that interfere with regular cellular functions, resulting in neuronal degeneration. The formidable ability of Garcinia indica and its metabolites to act as antioxidants makes them extremely potent in defending against these diseases.

4.7 Anti-fungal activity - Garcinia indica extract possesses antifungal and antibacterial characteristics, making it a promising option for bio preservation in the food industry and as a therapeutic remedy in cancer therapy. A recent study found that kokum rind extracts exhibited antifungal properties against Candida albicans, Penicillium sp., and Aspergillus flavus.

4.8 Antihistamine and Anti-inflammatory - In Ayurveda, kokum fruit is of great medicinal value. It has anti-helminthic (de-worming) and antihistamine properties. In addition, kokum also exhibits antihistamine and anti-inflammatory properties.

Pharmacological properties	Phytochemicals	References
Antibacterial	Garcinol, Iso garcinol and xanthochymol	[9, 10-11, 12, 13-15]
Anticlastogenic effect	Garcinol	[16]
Antidiabetic activities	Cyanidin 3-glucoside	[17-18]
Antineoplastic and Chemo preventive effects	Garcinol and Iso garcinol, Cyanidin-3-glucoside	[19,20, 21-22, 23-24,25,26]
Antifungal activity	1. Aqueous extract possesses antifungal action on Candida albicans and Penicillium sp 2. The chloroform extract from spent rinds inhibits the growth of and production of aflatoxin by Aspergillus flavus	[24, 27]
Anti-glycation activities	Garcinol	[28]
Anti-obesity activity	Hydroxycitric acid Cyanidin 3-glucoside	[29,30]
Antioxidant effects	Garcinol Cyanidin-3-glucoside	[28,31,16, 32, 27,33]
Cardioprotective effects	Cyanidin-3-glucoside	[34]
Gastroprotective effects	Garcinol	[28, 35-36]
Inhibition of carbonyl Content	Garcinol	[37]
Inhibitory effects on elastase and Hyaluronidase	Methanolic extract of kokum rind as well as the ethyl acetate and water fraction possess	[37]

	anti-hyaluronidase and anti-elastase activities *in vitro.*	
Inhibition of lipid peroxidation	Garcinol	[28,39,37]
Neuroprotection	Garcinol and Cyanidin-3-glucoside	[20,40-41]

Table 2: A summary of the nutraceutical properties of Kokum's bioactive components.

5. Objective:

The purpose of this review was to examine studies that supported the medical use of Kokum in asthma and to identify future research objectives.

Garcinia indica has anti-inflammatory, anti-allergic, and de-worming properties. It contains phenolic compounds that contribute to these properties. It is used as a traditional herbal medicine for diverse diseases in the Western Ghats of India, specifically in the states of Kerala, Karnataka, Maharashtra, and Goa. In the context of asthma, a chronic (long-term) illness that affects the lungs' airways, asthma is described as "a heterogeneous disease, usually characterized by chronic airway inflammation" by the Global Initiative for Asthma. According to the Global Initiative for Asthma (2017), it is recognized by the history of respiratory symptoms such as wheezing, shortness of breath, chest tightness, and coughing that change over time and in severity, in addition to variable expiratory airflow restriction.

As per the 2022 Global Asthma Report, 35 million individuals (about twice the population of New York) in India are affected by asthma. A comprehensive study and meta-analysis indicate that asthma affects 7.9% of children in India. Studies show that the prevalence of asthma ranges from 2.2% to 18.2%, with boys having a greater incidence (8.0%) than girls (5.9%). Asthma is also more common in mixed regions (7.9%), rural areas (5%), and urban areas (7.9%). Even though just 13% of people worldwide suffer from asthma, 42% of asthma-related fatalities worldwide are reported to occur in India, according to World Asthma Day 2022.

Asthma is initiated by multiple interactions between inflammatory cells and mediators. After being exposed to a triggering element, mast, macrophages, T-cells, and epithelial cells produce inflammatory mediators. This results in eosinophils and other inflammatory cells being drawn into the pulmonary tissues. These lead to hyperactivity of smooth muscles, hypersecretion of mucus, and lung damage. Additionally, according to Koda-Kimble (2009), the pathogenesis of asthma involves at least 27 cytokines and 18 chemokines. The primary cytokines that trigger allergy and asthma are Th1 cytokine interferon-gamma and Th2 lymphocyte cytokines [interleukin IL-4, IL-5, and IL-13] (Ngoc et al., 2005).

In general, the primary aims of asthma management are to achieve effective symptom control while minimizing the future risk of exacerbations, airflow restriction, and treatment adverse effects. Despite the availability of traditional medications, obtaining asthma control is deemed poor from a worldwide clinical perspective (Demoly et al., 2012; Price et al., 2014). According to Hawney et al. (2008) and Horne et al. (2007), one of the things causing suboptimal asthma management is poor adherence to asthma therapies. The intricacy of the treatment plan, inhaler technique issues, adverse events, and pharmaceutical costs are common medication-related causes of non-adherence (Bateman et al., 2008; Dima et al., 2015).

Among the main therapies of complementary and alternative medicine, herbal medications are often used by patients with asthma (Slader et al., 2006). Nevertheless, there is sometimes inadequate data to support the efficacy of these treatments for asthma. The purpose of this study was to examine and appraise the usage of kokum in asthmatic patients, as well as to identify future research goals by analyzing the available data.

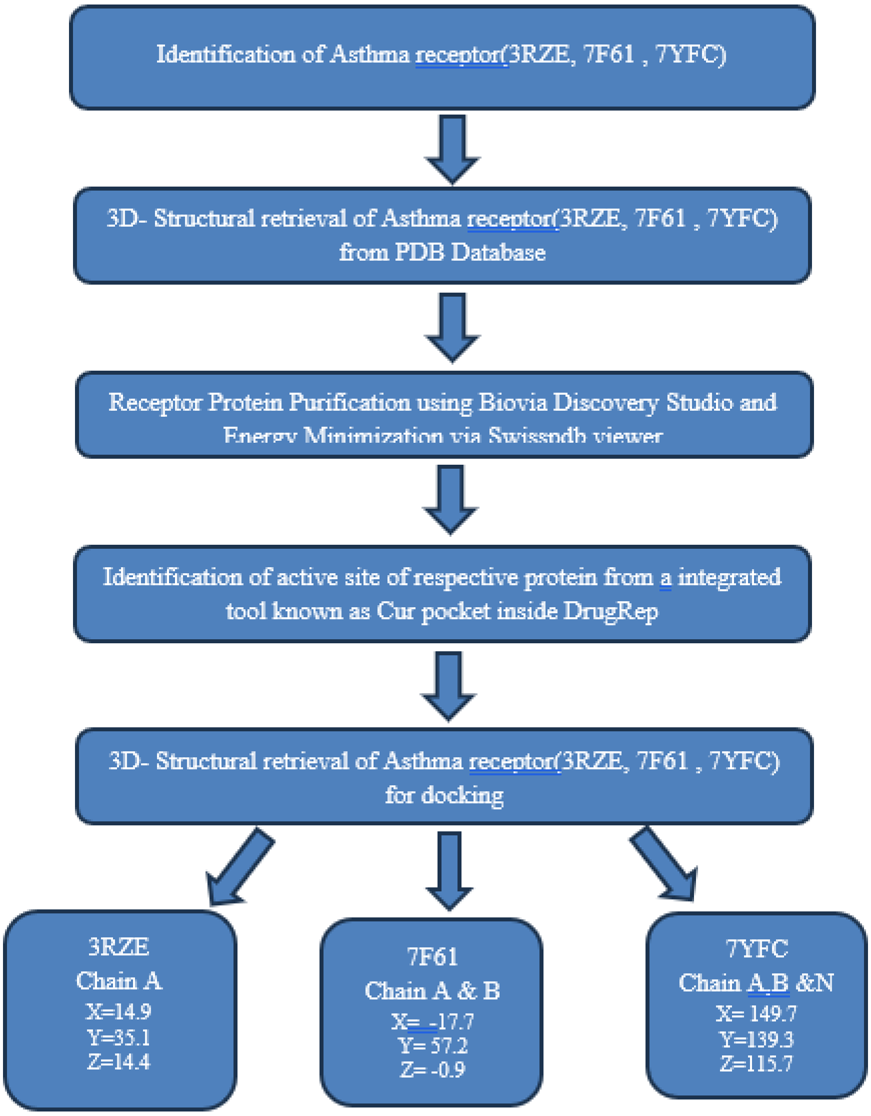
I. Methodology and Materials:

In Silico Docking Studies: In silico molecular docking studies were used to predict the mechanism of action of ligands and phenolic compounds on histamine proteins.

1.1.Retrieval of Protein Sequence and Structure

The RCSB PDB was utilized to extract high-resolution structures (3.10 Å) of the human histamine H1 receptor in complex with doxepin, (2.60 Å) of the human histamine receptor H3R in complex with antagonist PF03654746, and (3.00 Å) of the histamine-bound histamine H4 receptor and GQ complex. Using the apoprotein of the complex, extraneous molecules and undesired ions were removed to provide 3D files appropriate for docking simulation. In the context of ligand-molecular interactions, the co-crystallized inhibitor coordinates were considered. To reduce unfavorable interactions and stabilize the molecule, the process involved optimizing the apoprotein by improving the protein structure. The goal was to provide a more accurate representation of the original state of the protein for use in subsequent computational analysis.

An integrated tool inside DrugRep called Cur Pocket was used to automatically find possible binding pockets, and Auto Dock 4.0 was used for docking.



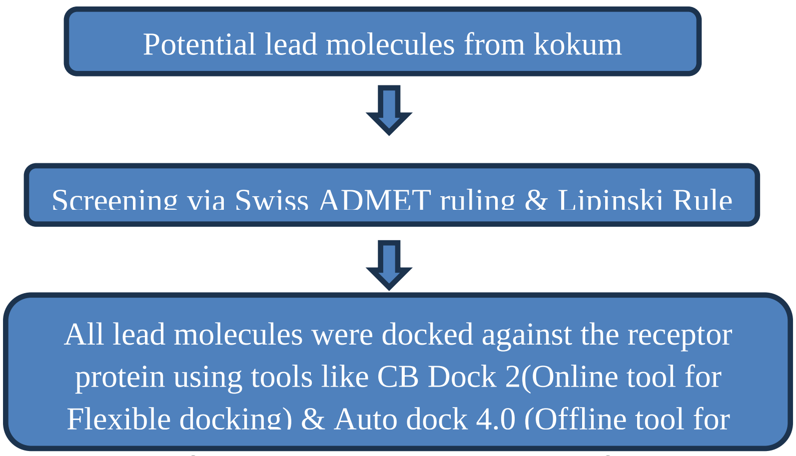
Flowchart Representation of Steps Involved in Protein receptor retrieval

1.2. Ligand retrieval and analysis

Using Marvin sketch, the 2-D structure of phenolic compounds was sketched, transformed into a 3-D structure, and then transcribed into the "PDB" format.

Phenolic Compounds	*Molecular Formulae*	*Molecular Weight (g/mol)*	*Molar Refractivity*	*H-Bond Acceptor*	*H-Bond Donor*	*MLOGP (Log P o/w)*	*Drug Likeness*
Anthocyanin	$C_{15}H_{11}O^{+}$	207.25	66.06	1	0	3.28	Yes;0 violation
Ascorbic Acid	$C_6H_8O_6$	176.12	35.12	6	4	-2.60	Yes;0 violation208.
Garcinol	$C_{38}H_{50}O_6$	602.80	180.06	6	3	3.78	Yes;1 violation; MW>500
Hydroxycitric Acid [HCA]	$C_6H_8O_8$	208.12	38.63	8	5	-2.27	Yes;0 violation
Iso garcinol	$C_{38}H_{50}O_6$	602.80	177.40	6	2	3.85	Yes;1 violation; MW>500
Xanthochymol	$C_{38}H_{50}O_6$	602.80	180.06	6	3	3.78	Yes;1 violation; MW>500

Table 3: List of metabolic phytochemical constituents of kokum



Flowchart Representation of Steps Involved in screening of ligand molecules of kokum and followed by Docking

1.3.Docking Analysis

One important metric for determining the strength and durability of an interaction is the binding free energy inside a protein-ligand complex. Auto Dock Vina (ADV), a component of the DrugRep database, explores the conformational space of the ligand within the protein binding site using a search algorithm. This approach finds the most thermodynamically favorable binding mode by evaluating the binding free energy of each produced posture. We emphasized the importance of hydrogen bonds in molecule identification and binding specificity by employing metrics like ΔG and hydrogen bond formation to identify potential candidates for ADME-sifted medicines.

Phenolic Compounds	3RZE [H1 RECEPTOR]		7F61 [H3 RECEPTOR]		7YFC [H4 RECEPTOR]	
	CBDock2 (kcal/mol)	Auto dock (kcal/mol)	CBDock2 (kcal/mol)	Auto dock (kcal/mol)	CBDock2(kcal/mol)	Auto dock (kcal/mol)
Anthocyanin	-8.5	-8.27	-9.8	-8.28	-8.1	-6.31
Ascorbic acid	-5.4	-3.78	-5.5	-5.02	-6.1	-3.90
Garcinol	-7.5	-1.17	-7.3	-8.29	-9.4	-4.86
HCA	-5.7	-3.01	-6.0	-3.01	-6.5	-4.53
Iso garcinol	-8.1	-4.29	-9.5	-8.19	-13.0	-4.43
Xanthochymol	-7.0	-2.31	-7.5	-4.91	-8.8	-3.78

Table 4: Experimental Data for Identified metabolic phytochemical constituents of kokum

6.3.2. Free Energy of Binding and Hydrogen Bonding Analysis in Interactions Between Ligands and Protein Receptors

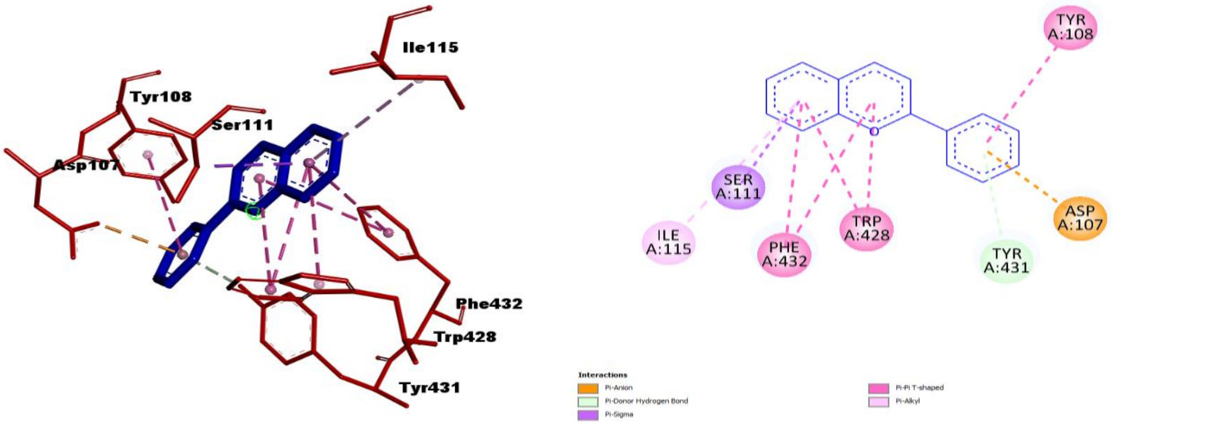


Fig 5: - Interaction between Histamin1 (3rze) and Anthocyanin metabolic compound of kokum (Garcinia Indica)

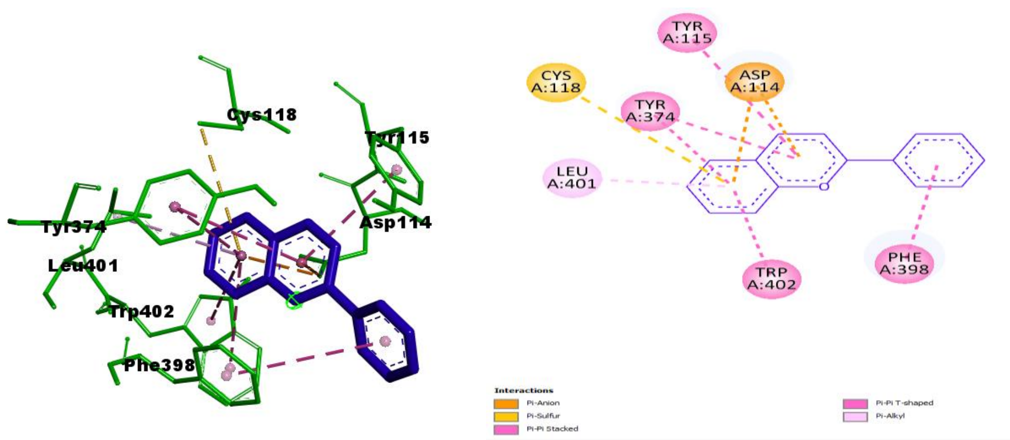


Fig 6: - Interaction between Histamin3 (7f61) and Anthocyanin metabolic compound of kokum (Garcinia Indica)

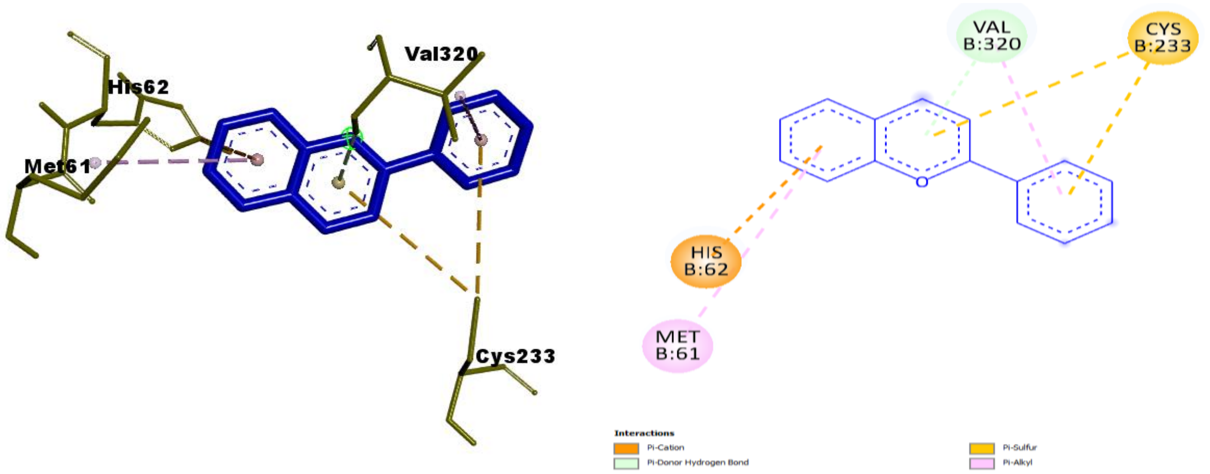


Fig 7: Interaction between Histamin4 (7yfc) and Anthocyanins metabolic compound of kokum (Garcinia Indica)

1.4.HIA and BBB permeability of metabolic compounds of kokum

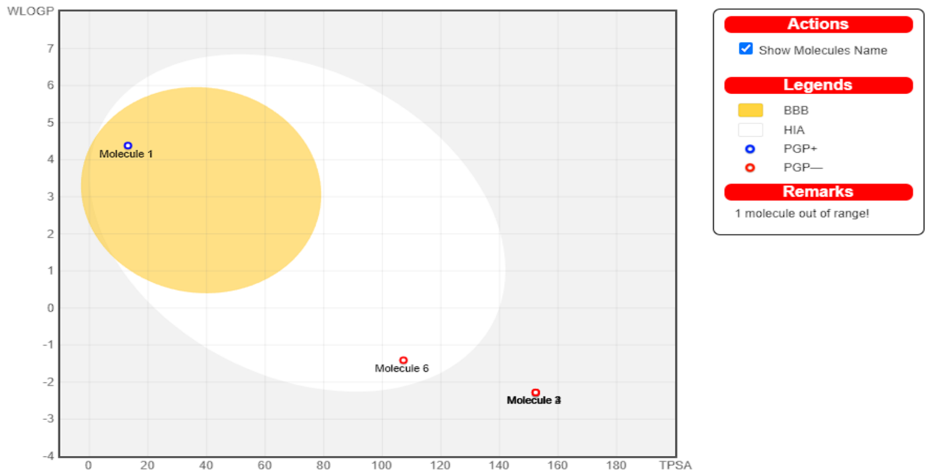


Figure 8: HIA and BBB permeability of metabolic compounds of kokum were assessed utilizing the Egan Egg model. (Complex 1 – Anthocyanin)

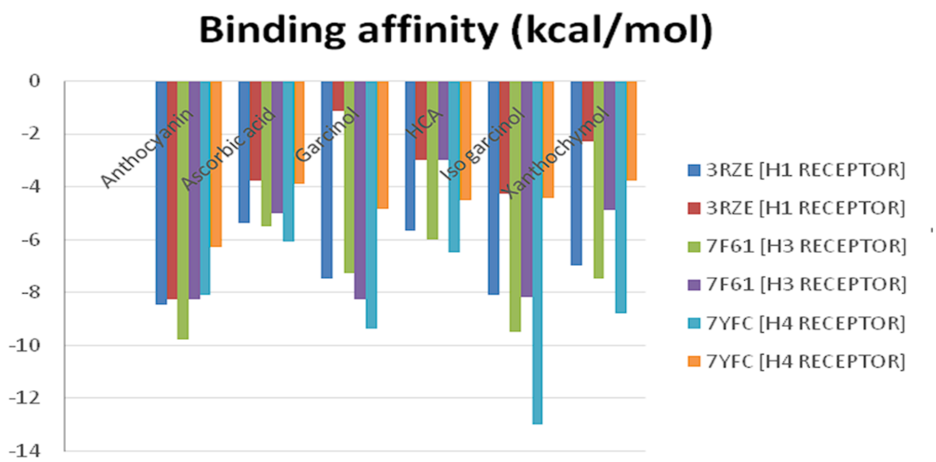


Fig 9: Graphical Representation of Docking Scores of various photochemical compounds against Asthma receptors.

6. Results:

This result shows that there is a presence of a binding site between the protein "3rze, 7f61, 7yfc and ligand-Anthocyanin, Ascorbic acid, Garcinol, Hydroxycitric Acid [HCA], Iso garcinol, Xanthochymol. The result of Lipinski rule suggests the analyzed compound as the best therapeutic drug. Docking study and in silico docking results proves the application of ligands as potential and natural therapeutic agent to treat asthma disease.

When docking was carried out among the three proteins and the ligands Anthocyanin, Ascorbic acid, Garcinol, Hydroxycitric Acid [HCA], Iso garcinol, Xanthochymol, the docked showed the binding affinity among them.

BENRALIZUMAB prevents the symptoms of asthma. It is prescribed when other asthma medications have not worked well enough. It works by decreasing inflammation of the airways, making it easier to breathe. But it holds Side effects that you should report to your care team as soon as possible: Allergic reactions or angioedema-skin rash, itching or hives, swelling of the face, eyes, lips, tongue, arms, or legs, trouble swallowing or breathing Side effects that usually do not require medical attention, headache, Sore throat.

In our Docking results, the ligands Anthocyanin, Ascorbic acid, Garcinol, Hydroxycitric acid, Iso-garcinol, Xanthochymol the docked outcome showed the binding affinity with three proteins (3rze, 7f61, 7yfc). Anthocyanin with Histamin1 (3rze) shows the highest docking score of -8.27 kcal/mol, Garcinol with Histamin3 (7f61) shows the highest docking score of -8.29 kcal/mol and Anthocyanin with histamin4 (7yfc) shows the highest docking score of -6.31kcal/mol.

7. CONCLUSION AND FEATURE ASPECTS-

In the study, molecular docking was carried out to determine the binding affinity of phytochemical which are found in kokum (Garcinia indica) with as Anti-Histamine proteins and to associate with its docking score. The outcomes are helpful for scheming and evolving a novel drug that has enhanced inhibitory activity against Asthma. This study should increase scholars' interest in new areas of investigations. This potential drug candidate awaits further justification by wet lab investigations for its accurate function as an antihistamine or asthma drug.

The future aspects on phytochemical analysis of kokum plants, specifically Garcinia indica, are promising, with ongoing research focusing on the identification and characterization of its bioactive compounds. The plant's pharmacological activities have been extensively studied, revealing a wide range of potential health benefits, including antioxidant, anti-obesity, anti-arthritic, anti-inflammatory, antibacterial, hepatoprotective, cardioprotective, antidepressant, and anxiolytic effect.

Future Directions

1. **In Silico Screening and Pharmacokinetic Studies**: The development of in silico tools and strategies for virtual screening, mode of action prediction, and advanced drug discovery will continue to play a crucial role in the exploration of kokum's phytochemicals.
2. **Pharmacological Activity of Garcinia indica:** Ongoing research will likely focus on the detailed analysis of the pharmacological activities of G. indica, including its antioxidant, anti-obesity, anti-arthritic, anti-inflammatory, antibacterial, hepatoprotective, cardioprotective, antidepressant, and anxiolytic effects.

3. **Phytochemical Evaluation and Antioxidant Potential**: The major phytochemical constituents present in G. indica, such as anthocyanins, hydroxycitric acid (HCA), garcinol, isogarcinol, ascorbic acid, and polyphenols, will continue to be evaluated for their antioxidant potential.
4. **Ethnobotanical Knowledge and Phytochemical Studies:** The ethnobotanical knowledge of kokum, particularly its consumption in India's western ghats as sharbat, will be further explored, and phytochemical studies will investigate the bioactive compounds responsible for its medicinal uses.
5. **Underutilized Tree with Medicinal Uses**: The underutilized tree Garcinia indica, commonly known as kokum, will continue to be studied for its phytochemistry and medicinal uses, highlighting its potential as a promising therapeutic agent to prevent various diseases

In summary, the future aspects on phytochemical analysis of kokum plants will involve the continued exploration of its bioactive compounds, their pharmacological activities, and the development of in silico tools for the discovery of new therapeutic agents.

BIBLIOGRAPHY

1. Khanashyam, Anandu Chandra & Gupta, Mathala. (2023). Potentials of kokum (Garcinia indica) - An underutilized Indian fruit as a functional food. 5.
2. Swami, S. B., Thakor, N. J., & Patil, S. C. (2014). Kokum (Garcinia indica) and its many functional components as related to the human health: A review. Journal of food research and technology, 2(4), 130-142.
3. Chate, M. R., Kakade, S. B., & Neeha, V. S. (2019). Kokum (Garcinia indica) fruit: a review. Asian Journal of Dairy and Food Research, 38(4), 329-332.
4. Jagtap, P., Bhise, K., & Prakya, V. (2015). A phytopharmacological review on Garcinia indica. International Journal of Herbal Medicine, 3(4), 02-07.
5. Ranveer, R. C., & Sahoo, A. K. (2017). Bioactive constituents of kokum and its potential health benefits. Nutrition and Food Toxicology, 1(6), 236-244.
6. Kongara Harshitha, Goluguri Achiritha, Sukarna Singha Roy, Calef T Suchen and Joshua T Suchen. Review based study on nutritional composition, health benefits and bioactive compounds of kokum. The Pharma Innovation Journal. 2022; 11(1S): 701-705. DOI: 10.22271/tpi.2022.v11.i1Sk.10213
7. Kushwaha P., Ahmad R., Srivastava A., Mehrotra S. Garcinia Indica Choisy: A Review on the Biological Activities of the Underutilized Medicinal Plant of Ayurveda Era J. Med. Res. 2023; 10(2): 60-64.
8. Koshak, A., Koshak, E., & Heinrich, M. (2017). Medicinal benefits of *Nigella sativa* in bronchial asthma: A literature review. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, *25*(8), 1130–1136. https://doi.org/10.1016/j.jsps.2017.07.002
9. Yamaguchi F., et al. “Antioxidative and Anti-Glycation Activity of Garcinol from Garcinia indica Fruit Rind”. Journal of Agricultural and Food Chemistry 48.2 (2000): 2320-2325.
10. Mishra A., et al. “Antioxidant activity of Garcinia indica (kokum) and its syrup”. Current Science 91.1 (2006): 90-93.
11. Sang S., et al. “Chemical studies on antioxidant mechanism of garcinol: Analysis of radical reaction products of garcinol with peroxyl radicals and their antitumor activities”. Tetrahedron 58.51 (2002): 10095-10102.
12. Rukachaisirikul V., et al. “An antibacterial biphenyl derivative from Garcinia bancana MIQ”. Chemical and Pharmaceutical Bulletin (Tokyo) 53.3 (2005): 342-343.

13. Yoshida K., et al. “Dietary garcinol inhibits 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in rats”. Cancer Letters 221.1 (2005): 29-39.
14. Varalakshmi KN., et al. “Antimicrobial and cytotoxic effects of Garcinia indica fruit rind extract.” American-Eurasian Journal of Agricultural & Environmental Sciences 7.2 (2010): 652-656.
15. Pasha C., et al. “Antisalmonella activity of selected medicinal plants”. Turkish Journal of Biology 33 (2009): 59-64.
16. Hong J., et al. “Effects of garcinol and its derivatives on intestinal cell growth: Inhibitory effects and autoxidation-dependent growth-stimulatory effects”. Free Radical Biology and Medicine 42.8 (2007): 1211-1221.
17. Negi PS and G K Jayaprakasha “Control of foodborne pathogenic and spoilage bacteria by Garcinol and Garcinia indica extracts and their antioxidant activity”. *Journal of Food Science Education* 69.3 (2006): 61-65.
18. Kirana Hand BP Srinivasan “Aqueous extract of Garcinia indica choisy restores glutathione in type 2 diabetic rats”. *Journal of Young Pharmacists* 2.3 (2010): 265-268.
19. Sahu A., et al. “Polyisoprenylated benzophenones from Garcinia pedunculata”. Phytochemistry 28. 4 (1989): 1233-1235.
20. Hong J., *et al*. “Effects of garcinol and its derivatives on intestinal cell growth: Inhibitory effects and autoxidation-dependent growth stimulatory effects”. *Free Radical Biology and Medicine* 42.8 (2007): 1211-1221.
21. Koeberle A., et al. “Identification of 5-lipoxygenase and microsomal prostaglandin E2 synthase-1 as functional targets of the anti-inflammatory and anti-carcinogenic garcinol”. Biochemical Pharmacology 77.9 (2009): 1513-1521.
22. Matsumoto K., *et al.* “Cytotoxic benzophenone derivatives from Garcinia species display a strong apoptosis-inducing effect against human leukemia cell lines”. *Biological Pharmaceutical Bulletin* 26.4 (2003): 569-571.
23. Iinuma M., et al. “Antibacterial activity of some Garcinia benzophenone derivatives against methicillin resistant Staphylococcus aureus”. Biological Pharmaceutical Bulletin 19.2 (1996):311-314.
24. Yoshida K., *et al.* “Dietary garcinol inhibits 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in rats”. *Cancer Letters* 221.1 (2005): 29-39.
25. Sasaki R., et al. “Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to down-regulation of retinol binding protein 4 expression in diabetic mice”. Biochemical Pharmacology 74.11 (2007): 1619-1627.
26. Ding M. et al. “Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemo preventive and chemotherapeutic activity”. The Journal of Biological Chemistry 281.25 (2006):17359-17368.
27. Tanaka T., et al. “Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F344 rats”. Carcinogenesis 21.6 (2000): 1183-1189.
28. Jena BS., et al. “Chemistry and Biochemistry of (-)-Hydroxycitric Acid from Garcinia”. Journal of Agricultural and Food Chemistry 50.1 (2002): 10-22.
29. Yamada Y., et al. “Chemistry, physiological properties, and microbial production of hydroxycitric acid”. Applied Microbiology and Biotechnology 75.5 (2007): 977-982.
30. Selvi, AT., et al. “Inhibition of growth and aflatoxin production in Aspergillus flavus by Garcinia indica extract and its antioxidant activity”. Food Microbiology 20.4 (2003): 455-460.

31. Cooke D., et al. "Effect of cyanidin-3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the ApcMin mouse model of intestinal carcinogenesis-relationship with tissue anthocyanin levels". International Journal of Cancer 119.9 (2006): 2213-2220.
32. Tsuda T., *et al.* "Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice." *The Journal of Nutrition* 133.7 (2003): 2125-2130.
33. Elisia I and Kitts DD. "Anthocyanins inhibit peroxyl radical-induced apoptosis in Caco-2 cells". Molecular and Cellular Biochemistry 312 (1-2) (2008): 139-145.
34. Bala Subramanyam K., et al. "Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression". The Journal of Biological Chemistry 279.32 (2004): 33716-33726.
35. Chatterjee A., et al. "The bactericidal effects of Lactobacillus acidophilus, garcinol and Protykin compared to clarithromycin, on Helicobacter pylori". Molecular & Cellular Biochemistry 243.1-2 (2003): 29-35.
36. Xu JW., et al. "Upregulation of endothelial nitric oxide synthase by cyanidin-3-glucoside, a typical anthocyanin pigment." Hypertension 44.2 (2004): 217-222.
37. Kolodziejczyk J., et al. "Effects of garcinol and guttiferone K isolated from Garcinia cambogia on oxidative/nitrative modifications in blood platelets and plasma". Platelets 20.7 (2009): 487-492.
38. Pan MH., et al. "Induction of apoptosis by garcinol and curcumin through cytochrome Corelease and activation of caspases in human leukemia HL-60 cells". Journal of Agricultural and Food Chemistry 49.3 (2001): 1464-1474.
39. Sahasrabudhe A., et al. "Anti-hyaluronidase, anti-elastase activity of Garcinia indica." International Journal of Botany 6.3 (2010): 299-303.
40. Chen PN., et al. "Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line". Cancer Letters 235.2 (2006): 248-259.
41. Krishnamurthy N (1984). Chemical and technological studies on colouring matters from natural sources for use in foods. *Ph.D. Thesis, Mysore University.*
42. Krishnamurthy N, Lewis YS and Ravindranatha B (1982).Chemical constitution of Kokum fruit – rind. *Journal of Food Science and Technology*, 19: 97-100
43. Chandran, M. S. (1996). Nature watch: The kokum tree. *Resonance*, *1*(1), 86-89
44. Chemexcil (1992). Selected medicinal plants of India. Basic Chemicals,Pharmaceutical and Cosmetic Export Promotion Council, Bombay 400039, India.
45. Nayak CA and Rastogi NK (2010). Forward osmosis for the concentration of anthocyanin from *Garcinia indica Choisy. Separation and Purification Technology*, 71: 144-151.
46. Nayak CA, Rastogi NK and Raghavarao KSMS (2010).Bioactive constituents present in Garcinia indica Choisy and its potential food applications: A review.International Journal of Food Properties, 13(3): 441-453.
47. Sang S, Pan MH, Cheng X, Bai N, Stark RE, Rosen RT, Lin-Shiau, SY, Lin JK and Ho CT (2001). Chemical studies on antioxidant mechanism of garcinol:analysis of radical reaction products of garcinol and their antitumor activities. Tetrahedron, 57:9931-9938.
48. Delgado-Vargas, F., Jimenez, A. R., & Paredes-Lopez, O. (2000). Critical reviews in food science and nutrition. Natural pigments: Carotenoids, anthocyanins, and betalains—characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, *40*(3), 173-289.

49. Bridle, P., & Timberlake, C. F. (1997). Anthocyanins as natural food colours—selected aspects. *Food chemistry*, *58*(1-2), 103-109.
50. Jagtap, P., Bhise, K., & Prakya, V. (2015). A phytopharmacological review on Garcinia indica. *International Journal of Herbal Medicine*, *3*(4), 02-07.
51. Lewis YS, Neelkantan S and Murthy C (1964). Acid in Garcinia Cambogia. Current Science, 33: 82-83.
52. Rai, S. R., & Sayeed, A. 5. Kokum-The Superfood of India.
53. Barve, K. (2021). Garcinol enriched fraction from the fruit rind of Garcinia indica ameliorates atherosclerotic risk factor in diet induced hyperlipidemic C57BL/6 mice. *Journal of Traditional and Complementary Medicine*, *11*(2), 95-102.
54. Munjal, K., Ahmad, S., Gupta, A., Haye, A., Amin, S., & Mir, S. R. (2020). Polyphenol-enriched fraction and the compounds isolated from Garcinia indica fruits ameliorate obesity through suppression of digestive enzymes and oxidative stress. *Pharmacognosy Magazine*, *16*(70).
55. Dhamija, I., Parle, M., & Kumar, S. (2017). Antidepressant and anxiolytic effects of Garcinia indica fruit rind via monoaminergic pathway. *3 Biotech*, *7*, 1-12.
56. Antala, B. V., Patel, M. S., Bhuva, S. V., Gupta, S., Rabadiya, S., & Lahkar, M. (2012). Protective effect of methanolic extract of Garcinia indica fruits in 6-OHDA rat model of Parkinson's disease. *Indian Journal of Pharmacology*, *44*(6), 683-687.