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5. Kokum-The Superfood of India

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5.1 Introduction:

Garcinia indica, commonly known as Kokum, is an ancient fruit and a plant native to certain regions of India. It is mostly grown along the western peninsular coastal regions and the adjacent Western Ghats in Maharashtra's South Konkan region, Goa, Karnataka, and Kerala, as well as parts of Eastern India's West Bengal, Assam, and North Eastern Hill region. (Senthil et.al.,2014) A broad variety of kokum trees can be found in the Western Ghats, a great source of biodiversity for various plant species.

Kokum is a tree that grows in evergreen and semi-evergreen woods and in-home gardens. The tree bears fruit every year during the warm months of March to May (Chate et al., 2019). Kokum is one of the most important indigenous tree spice crops belonging to the Mangosteen family (Clusiaceae).

It is a sticky, circular-shaped fruit, which is green when raw and red to dark purple when fully ripe. Kokum has been used as an Ayurvedic medicinal herb for curing many ailments. It also has a diverse application in culinary, pharmaceutical, neutraceutical and industrial aspects.

Table 5.1: Chemical Composition of Kokum Fruit (Chate et al., 2019)

NUTRIENTS	QUANTITY (%)
Moisture	80
Protein (N x6.25)	1.92
Crude fat	10
Crude fiber	14.28
Total ash	2.57
Carbohydrates (by difference)	35
Starch	1
Pigments	2.40

NUTRIENTS	QUANTITY (%)
Tannin	2.85
Pectin	5.71
Ascorbic acid	0.06
Hydroxyl citric acid	22.80

5.2 Components of Kokum:

Kokum fruit contains many bioactive compounds which exhibits antioxidant, anti-mutagenic, anti-fungal and anti-bacterial and anti-inflammatory properties. The three major bioactive compounds present are namely anthocyanin, hydroxycitric acid and garcinol, all of these possesses nutraceutical properties. (Ranveer et.al.,2017)

A. Anthocyanins:

The two principal anthocyanin pigments found in Kokum are cyanidin-3-glucoside and cyanidin-3-sambubioside. In fruits, anthocyanins make up about 2.4 percent of the biomass. These pigments are water-soluble and free radical scavengers. Anthocyanins are a type of flavonoids that imparts fruits their red and purple hues.

The skeleton of anthocyanins is C-15, with a chromane ring in position 2 and another aromatic B-ring in position 3. (C6-C3-C6). One or more sugar molecules are commonly connected to the basic structure's numerous hydroxylated sites. Anthocyanins are phenyl-2-benzopyrilium salts with substituted glycosides (anthocyanidins).

Anthocyanidin's fundamental structure is as follows: -H, -OH, or -OCH3 R = -H, -OH, or -OCH3 (Vargas et al., 2000). Kokum's cyanidin-3-glucoside has hydroxyl groups at the appropriate places and a glycosidic bond at position 3. The other primary pigment, cyanidin-3-sambubioside, is structurally identical to glucose but contains the disaccharide sambubioside instead of glucose. Anthocyanins are antioxidants that prevent ascorbic acid oxidation, scavenge free radicals, inhibit oxidative enzymes, and lower the risk of cancer and heart disease. With a saturated 2,3- double bond, the 3' and 4' –OH in the B-ring dictate radical scavenging capacity.

When present as glucosides, the antioxidant activity increases as the hydroxyl groups in the B-ring increase. It was discovered that the number of hydroxyl groups in the B-ring boosted the radical scavenging activity and reduction capacity. Anthocyanins-metal chelation prevents ascorbic acid oxidation by the 3' and 4' –OH groups (Azevedo et al., 2010).

Lipid peroxidation is also influenced by anthocyanins. They are more effective than tocopherol in preventing lipid peroxidation. Anthocyanins also act as scavengers for the oxidants OH and O2. Bioflavonoids like leucoanthocyanidins, catechins, and flavonols, as well as anthocyanins like cyanidin-3-glucoside, have been proven to increase capillary permeability and strength, speed up ethanol metabolism, and minimize inflammation and eremitic responses (Vargas et al., 2000).

B. Hydroxycitric Acid:

In kokum, hydroxycitric acid (HCA) is a significant acid. On a dry basis, kokum can contain up to 23% HCA. The majority of the HCA is found in the leaves and rinds, with a small amount in the form of HCA lactone. The second and third carbon atoms of HCA contain hydroxyl groups. Because HCA has two asymmetric carbons, it possesses two diastereoisomers. Garcinia species contain the most (-)- hydroxycitric acid of the four. During evaporation or concentration, free HCA is easily transformed into HCA lactone. The enzyme ATP: citrate lyase is inhibited by (-)-HCA (ATP: citrate oxaloacetate lyase). This enzyme is important in the production of fatty acids from carbohydrates. The cleavage of citrate to acetyl-CoA and oxaloacetate is catalyzed by this enzyme (Ranveer et al., 2017). Acetyl-CoA is the final source of carbon for fatty acids, and it is a crucial component in the production of lipids from carbohydrates. As a result, (-)-HCA plays a key role in controlling fatty acid synthesis by reducing acetyl-CoA availability. The fact that HCA inhibits ATP: citrate lyase so well aids in the research of the citrate cleavage reaction (Yamada et al., 20007). HCA has been shown to promote fatty acid production in some circumstances. HCA suppresses lipogenesis only when the citrate cleavage enzyme produces cytoplasmic acetyl-CoA; otherwise, if an alternative source of acetyl-CoA, such as acetate, is available, it activates fatty acid synthesis. HCA is also said to be helping in anti-obesity because it modulates the ATP: citrate lyase enzyme and hence the citrate cleavage reaction. It is also recognised as a weight-controlling agent due to its regulatory impact. HCA can be utilised to boost carnitine palmitoyl transferase activity (CPT 1). CPT 1 is a fat-burning and weight-loss rate-limiting factor. HCA lowers acetyl-CoA production, which reduces malonyl-CoA production. Malonyl-CoA acts as a CPT 1 inhibitor. HCA works as a weight-loss agent by lowering malonyl-CoA formation (Yamada et al., 20007).

C. Garcinol:

Garcinol, a polyisoprenylated benzophenone derivative found in kokum fruit, accounts for 1.5 percent of its weight. Garcinol is a yellow-colored fat-soluble pigment discovered in small amounts in the rinds of kokum (Jena et al., 2002). Garcinol acts as an active antioxidant. Camboginol, a tri-isoprenylated chalcone, is another name for garcinol. It has a diketone moiety and so resembles curcumin, again a well-known antioxidant (Sahu et al., 1989).

Garcinol's anti-cancer, anti-ulcer, anti-oxidative, and anti-glycation properties have all been investigated (Nayak et al., 2010). Garcinol can scavenge alkyl-peroxyl radicals, resulting in the hydroperoxy derivatives of garcinol, cambogin, and isogarcinol. Isogarcinol is a strong antioxidant with biological properties comparable to garcinol. These chemicals can cause apoptosis in human leukaemia HL-60 cells, as well as suppress NO radical production and LPS-induced iNOS gene expression. As a result, garcinol has been found to have superior anticancer properties. Garcinol has been demonstrated to have antioxidant activity and radical scavenging activity against the hydroxyl radical, methyl radical, and superoxide anion in the H2O2-NaOH-DMSO system. The emulsified garcinol suppresses superoxide anion and has three times the free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. By modulating arachidonic acid metabolism, garcinol has been found to have an antioxidant effect against arachidonic acid metabolism and NO radical production. Carcinogenesis and inflammation are linked to faulty arachidonic acid

metabolism and the production of NO radicals. Garcinol has been found to prevent the buildup of NO radicals in LPS-induced inflammatory mediators like iNOS and COX-2 (Ranveer et al., 2017). Garcinol can also be an excellent alternative to conventional antibiotics and also operates as an anti-tumor agent by inducing apoptosis through the activation of caspases (Sahu et al., 1989).

5.3 Pharmacological and Biological Properties of Kokum:

A. Anti-Cancer Activity:

Cancer, the uncontrolled growth and spread of abnormal cells, results from the accumulation of numerous sequential mutations and alterations in nuclear and cytoplasmic molecules. It is important to develop effective preventative and/or could potentially be both effective against cancer cell growth and relatively non-toxic.Garcinol, a yellow-coloured, fat-soluble pigment discovered in the rinds of Kokum, contains 1.5 percent polyisoprenylated benzophenone derivative found in the rinds of kokum at a level of 2-3 percent (Yamaguchi et al., 2000). Recent evidence supports that bioactive compounds found in *Garcinia indica* like xanthones, flavonoids and benzophenones have therapeutic benefits attributable to their pleiotropic effects including downregulation of survival signaling and simultaneous activation of multiple death pathways in cancer cells (Ahmad et al., 2010).

B. Antioxidant Activity:

Antioxidants are micronutrients that can neutralize free radicals or their actions. Free radicals have been implicated in the etiology of several major human diseases, including cancer, cardiovascular diseases, diabetes and arthritis. Due to the recent trends in nutrition towards the development of healthy foods in the form of ‘functional foods, one of the important properties in a dietary component is considered to be its antioxidant property (Mishra et al. 2006).

Kokum contains other naturally occurring compounds with potential antioxidant properties such as citric acid, malic acid, polyphenols, carbohydrates, anthocyanin flavonoids and ascorbic acid (Rastogi and Nayak, 2010; Einbond et al. 2004). Garcinol,a polyisoprenylated benzophenone, purified from *Garcinia indica* fruit rind is a potent antioxidant. *Garcinia indica* contains 2 to 3 % of water-soluble red colour pigments. Anthocyanin is a major water-soluble pigment present in the kokum. Two major anthocyanin pigments cyanidin-3-glucoside and cyanidin-3-sambubioside are usually present in the ratio of 4:1. Kokum contains a high concentration of anthocyanins (2.4 g/100 g) compared to other natural sources (Nayak et al. 2010). *Garcinia indica* bark exudates showed their total phenol and xanthone content as 53.43g/100g and 32.42 g/100g respectively, revealing it as a potential source of natural antioxidants (Chate et al., 2019).

C. Anti-Obesity Activity:

Kokum fruit juice is very acidic with a pH of 1.5 to 2.0 and contains large amounts of acids. A major portion of organic acids in kokum is hydroxycitric acid (HCA) (1, 2 dihydroxy propane-1, 2, 3- tricarboxylic acid). Rinds of *Garcinia indica* contain about 20-30% of

hydroxycitric acid (HCA) dry basis (Swami et al., 2014). Hydroxycitric acid (HCA) has been patented for use as a hypocholesterolaemic agent because of its anti-obesity activity. (Mir et.al.,2020) Hydroxycitric acid and its derivatives are useful in the treatment of obesity. It suppresses fatty acid synthesis, lipogenesis and food intake and induces weight loss. Besides hydroxycitric acid (HCA), *Garcinia indica* fruit juice also contains malic acid, citric acid and tartaric acid (Parthasarathy et al., 2012).

Furthermore, utilising the cholesterol-induced hyperlipidemic paradigm, the methanolic extract of the dried fruit of kokum demonstrated excellent anti-hyperlipidemic action in rats. Total cholesterol, triglycerides, LDL-C, and VLDL-C levels all decreased significantly, while HDL-C levels increased.

Many studies have shown that consuming kokum's hydroxy citric suppresses lipogenesis and decreases body weight. In vitro investigations of rat adipocytes treated with cyanidin 3-glucoside revealed an increase in adipocytokine production and up-regulation of adipocyte-specific gene expression without activation of PPAR. Furthermore, in vivo investigations revealed an increase in adiponectin gene expression in white adipose tissue. Iso-garcinol also exhibited lipase inhibitory and anti-obesity effects (Tsuda et al., 2010).

D. Anti-Ulcer Activity:

Garcinia indica aqueous and ethanol extracts were tested for ulcer-protective efficacy against indomethacin-induced ulcerogenesis and HCl/ethanol-induced gastric lesion. (Panda and Khambat, 2014). In the HCl/ethanol and indomethacin produced gastric lesion rat models, oral treatment of the aqueous and ethanol extracts of Garcinia indica fruit rind at a concentration of 500 mg/kg resulted in a substantial decrease in ulcer index (Yamaguchi et al., 2000).

E. Antifungal Activity:

Garcinia indica extract exhibits antifungal characteristics and has the potential to be used as a bio preservative in food applications as well as a cancer therapeutic agent. According to one study, kokum rind extracts have antifungal properties against *Candida albicans, Penicillium sp.,* and *Aspergillus flavus* (Varalakshmi et al. 2010).

F. Anti-Bacterial Activity:

Hexane and benzene extracts of kokum rinds and its active ingredient garcinol have strong antibacterial action on their own. It also increased the effectiveness of clarithromycin against *H. pylori* (Chatterjee et al., 2005). Even kokum leaf extract inhibits the pathogenic microorganisms *Salmonella typhi, Salmonella paratyphi A,* and *Salmonella typhimurium*.

The greatest antibacterial activity has been observed for aqueous extract of kokum rind against *Bacillus subtilis,* followed by *Escherichia coli, Enterobacter aerogenes,* and *Staphylococcus aureus.* Garcinol, iso-garcinol, and xanthochymol were found to have an inhibitory impact on the development of methicillin-resistant *S. aureus* (Jagtap et al., 2015).

G. Anti-Aging Activity:

The elasticity of the skin decreases with age owing to elastase enzymes, causing drooping, and hyaluronic acid in the skin also declines, causing the skin to become dry and wrinkled. As a result, matrix metalloproteins must be conserved by limiting the action of matrix metalloproteinases. Because of the presence of the phenolic group, garcinol found in the fruit rinds of *Garcinia indica* is a strong antioxidant. In addition, several preparations of *Garcinia indica* have an essential function in anti-aging (Chate et al., 2019). Because of their UV light absorption qualities, kokum pigments are effective in skin problems and skin care. (Kureel et al., 2009) Kokum has anti-hyaluronidase and anti-elastase properties that benefit skincare (Sahasrabudhe et al., 2010).

5.4 Neuroprotective Effects:

In rats, methanolic extract of kokum fruit shown considerable neuroprotective potential against 6-OHDA, indicating antiparkinson action. (Antala et al.,2012) Garcinol also inhibited the production of anti-inflammatory mediators generated by LPS. In LPS-treated astrocytes, iNOS and COX-2 were inhibited, and nitric oxide buildup was avoided. It was also discovered to possess anticholinesterase effects. Cyanidin-3-glucoside inhibits neurite outgrowth and neurofilament protein expression, suggesting its neuroprotective potential (Lim et.al.,2021). *Garcinia indica* has shown to have a significant positive effect as an antidepressant and anxiolytic effect. (Dhamija, et.al.,2017)

A. Hypoglycemic Activity:

In both acute and chronic investigations, kokum whole fruit extract effectively reduced fasting blood glucose levels in streptozotocin-induced hyperglycemic rat. An acute dose of 400 mg/kg of the aqueous extract enhanced oral glucose tolerance, indicating anti-hyperglycemic action. (Malli et al.,2017; Deore et al.,2018) Garcinol isolated from the rind of *Garcinia indica* has been shown to have a significant glycation inhibitory effect, suppressing protein glycation in a bovine serum albumin/fructose system (Jagtap et al., 2015).

B. Anti-Inflammatory Activity:

The anti-inflammatory activity of kokum rind aqueous and ethanolic extracts was examined utilising the carrageenan-induced paw edema model. In the acute testing, both extracts significantly reduced inflammation. Furthermore, a considerable decrease in acid phosphate and alkaline phosphate lysosomal enzymes shows its anti-inflammatory efficacy (Swami et al., 2014; Pandey, 2009).

C. Appetite-Suppressing Activity:

HCA increases the levels of satiety. When a person is satisfied with his/her very first bites, the easier it is to eat less because they will feel that they have already had enough to eat. HCA's ability to suppress appetite is due to an increase in serotonin levels in the brain.

HCA, the primary acid in the fruit rind *Garcinia indica* has been reported as the active ingredient in inhibiting ATP citrate lyase. ATP citrate lyase, which is an extramitochondrial enzyme catalyzing the cleavage of citrate to oxaloacetate and acetyl-CoA, is inhibited by HCA. Thus, the availability of two-carbon units required for the initial steps of fatty acid and cholesterol biosynthesis during carbohydrate feeding becomes limited. As a result, the consumed carbon source is diverted to glycogen synthesis in the liver. A signal is then sent to the brain due to this metabolic alteration, resulting in a rise in serotonin level concomitant with a reduced appetite. Several reports have supported the serotonin regulation of HCA (Jagtap et al., 2015).

Table 5.2: Biological activities of Kokum and their observations (Padhye et al., 2017).

ACTIVITY	OBSERVATIONS
Anti-oxidant	Efficient scavenging of free radicals. Inhibition of NO and H202 production. Inhibition of NO and iNOS Generation. Inhibition of iNOS and COX-2 expression. Inhibition of NO accumulation.
Anti-bacterial	Activity against methicillin-resistant *Staphylococcus aureus.* Efficient killing of *Helicobacter pylori.*
Anti-cancer	Chemoprevention of colon tumorigenesis. Induction of caspase-3-mediated apoptosis. Loss of mitochondrial potential and activation of caspase 3. Induction of apoptosis. Inhibition of tongue carcinogenesis. Modulation of arachidonic acid metabolism and inhibition of STAT-I. Selective killing of colon cancer cells. Induction of apoptosis and inhibition of cell invasion.

Table 5.3: Summary of reported nutraceutical activities of bioactive compounds of Kokum (Ranveer et al., 2017)

PHARMACOLOGICAL PROPERTIES	PHYTOCHEMICALS
Antibacterial	Garcinol, iso-garcinol and xanthochymol.
Anticlastogenic effect	Garcinol
Antidiabetic activities	Cyanidin 3-glucoside
Antineoplastic and Chemo-preventive effects	Garcinol and iso-garcinol, Cyanidin 3-glucoside

PHARMACOLOGICAL PROPERTIES	PHYTOCHEMICALS
Antifungal activity	1. Aqueous extract possess 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.*
Anti-glycation activities	Garcinol
Anti-obesity activity	Hydroxycitric acid and Cyanidin 3-glucoside
Antioxidant effects	Garcinol, Cyanidin 3-glucoside
Cardioprotective effects	Cyanidin-3-glucoside
Gastroprotective effects	Garcinol
Inhibition of carbonyl Content	Garcinol
Inhibitory effects on elastase and Hyaluronidase	Methanolic extract of kokum rind as well as the ethyl acetate and water fraction possess anti-hyaluronidase and anti-elastase activities in vitro.
Inhibition of lipid peroxidation	Garcinol
Neuroprotection	Garcinol and Cyanidin-3-glucoside

5.5 Conclusion:

A functional food's positive effects should be maintenance of health or well being or a reduction in the chance of contracting a certain ailment. Kokum fits this criterion in several ways because of the presence of vital bioactive components which demonstrates physiological and psychological effects beyond the nutritional benefits.

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