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REVIEW ARTICLE……!!!

# METHODS FOR ISOLATION OF BIOACTIVE CONSTITUENTS FROM *Garcinia indica choisy* AND ITS MEDICINAL IMPORTANCE: A REVIEW

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**KEYWORDS:**

*Garcinia indica* choisy, Hydroxy citric acid, Anthocyanin, Garcinol, Anti-oxidant.

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## ABSTRACT

*Garcinia indica* (dried rind known as 'kokum'), a tropical fruit, can be viewed as a wonder berry that has a pleasant, tangy-sweet taste and a myriad of health benefits. Kokum fruit is a potential source of Hydroxy citric acid, anthocyanins and a polyisoprenylated benzophenone derivative, garcinol. Recently, hydroxyl citric acid has been found to be used as a potent metabolic regulator of obesity and lipid abnormalities in mammalian system. Kokum is loaded with B-complex vitamins, and minerals like potassium, manganese and magnesium, which help in control heart rate and blood pressure, offering protection against stroke and coronary heart diseases. This versatile fruit has been used to counter digestive problems such as indigestion, flatulence, acidity and constipation. Kokum fruit possess useful antioxidant, chelating, anti-cancer, anti-fungal, anti-inflammatory, anti-bacterial, cardio protective and anti-ulcer activities. The review highlights the bioactive constituents present in kokum fruit and also discusses its various isolation methods and its pharmaceutical applications.

**INTRODUCTION:**

Kokum (*Garcinia indica* choisy) trees are found in humid tropical regions of Western Ghats of India. Most useful part of the plant is the fruit of Kokum. This fruit is of commercial importance owing to its enormous medicinal properties. Genus Garcinia of the Clusiaceae family includes around 200 species of trees or shrubs, of which *Garcinia indica* is the most common. Kokum is a berry with fleshy endocarp whose seeds, rind, juice and pulp have a myriad of health benefits. Kokum fruit is a popular condiment used in several states of India for making vegetarian and non-vegetarian 'curry' preparations, including the popular 'solkadhi'[1,2]. A healthy soft drink is made from Kokum to relieve sunstroke, due to its heat neutralizing property. Kokum fruits are squeezed in sugar syrup to make a soft drink, named as 'Amrutkokam'[3], which is quite popular during summer season. In Ayurveda, Kokum is traditionally used for edema, rheumatism, delayed menstruation, constipation, bowel complaints, intestinal parasites, skin rashes and burns. Kokum juice is used as a weight loss supplement, since it is anorectic. It is used as hepato-tonic, cardiotonic, anti-tumor and as a cure for bleeding piles. This plant is also pharmacologically studied for chelating, free radical scavenging, anti-bacterial, anti- fungal, anti-cancer, anti-inflammatory, anti-obesity and anti- ulcer activities. The rind is processed for preparation of syrup, amsul, Kokum powder etc.

**BOTANICAL STUDY:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Malpighiales |
| Family | : | Clusiaceae |
| Subfamily | : | Clusioideae |
| Tribe | : | Garcinieae |
| Genus | : | *Garcinia* |
| Species | : | *Garcinia indica* Choisy |

**GEOGRAPHICAL DISTRIBUTION:**

Kokum is a tropical evergreen tree of moderate to large size. It is found at an altitude of about 800 meters from sea level. It is a slender tree with drooping branches. It grows to a height of 15-20m. The canopy is dense with green leaves. It is a native of the Western Ghats region of India. It is distributed throughout Konkan, Goa, North & south kanara, North Malabar, Coorg &Wynad as well as in Westt Bengal and Assam. It is androdioceious tree producing male and bisexual flowers on separate plants [4].

## CHEMICAL CONSTITUENTS

Garcinia is a rich source of active compounds including garcinol, xanthochymol, isoxanthochymol and Hydroxycitric acid. These are flavonoids, benzophenones, xanthones, lactones and phenolic acids [5]. The fruits contain citric acid, acetic acid, malic acid, ascorbic acid, hydroxycitric acid and garcinol. The major constituent of Kokum rind is garcinol, a polyisoprenylated benzophenones, isogarcinol and camboginol. Garcim-1, Garcim-2 and cambogin are the chief oxidative products of garcinol, along with isogarcinol, gambogic acid, mangostin, clusianone, macurin, oblongifolin (A, B, C), guttiferone (I, J, K, M, N). The rind of ripe Kokum fruits consists of hydroxyacetic acid and hydroxycitric acid. It also contains 2.4% pigment as a mixture of two anthocyanins namely, cyanidin-3-sambubioside and cyanidin-3-glucoside in the ratio 4:1. Studies have shown that the fresh rind of Kokum contains 80% moisture, 2% protein, 2.8% tannin, 5% pectin, 14% crude fiber, 4.1% total sugars, 1.4% fat, 2.4% pigment, 22% hydroxycitric acid, 0.06% ascorbic acid [6]. Kokum leaves are reported to contain L-leucine, 75% moisture, protein 2.3g, fat 0.5g, fiber 1.24g, carbohydrates 17.2g, iron 15.14mg, calcium 250mg, ascorbic acid 10mg and oxalic acid 18.10mg per 100g . Hydroxycitric acid lactone and citric acid are present in leaves and rinds in minor quantities[7]. Kokum seeds are rich in glycerides of stearic acid (55%), oleic acid (40%), palmitic acid (3%), linoleic acid (1.5%), hydroxyl capric acid (10%) and myristic acid (0.5%).

## METHODS FOR ISOLATION OF BIO ACTIVE INGREDIENTS

Garcinol, Hydroxyl citric acid, Anthocyanins are the major bio-active ingredients present in the *Garcinia indica.*

## ISOLATION OF GARCINOL

***Purification and Structural Analysis of Garcinol***—Garcinol was prepared from *Garcinia indica* fruit rind. In brief, *G. indica* dried fruit (Kokum) rind was extracted with ethanol, and the extract was fractionated by ODS (octadecyl silica) column chromatography eluted stepwise with 60–80% aqueous ethanol. The fractions containing garcinol were concentrated and dried in vacuum. The residue was dissolved in hexane, and the solution was cooled at 5 °C for 2 days. Yellow amorphous precipitate was collected from the solution and washed with cold hexane and recrystallized at room temperature[8]. Pale yellow needle crystals were obtained from the solvent, which were identified as garcinol from the following spectral data: mp 126 °C; Optical rotation at 30–135 ($CHCl_3$); UV in EtOH (log ϵ) 367 (3.84) and 250 (4.05) nm; IR 3200–3500, 1730, 1640 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.95 (1H, dd, J = 9.0 and 2.0 Hz), 6.91 (1H, d, *J* = 2.0 Hz), 6.60 (1H, d, *J* = 9.0 Hz), 4.96, 5.06, 5.10 (1H each, t, *J* = 5.0Hz), 4.40 (d, *J* = 15.0 Hz), 2.80–1.46 (m, 12H,

methylene and methyne), 1.78, 1.74, 1.69, 1.62, 1.59, 1.56, 1.21, 1.05 (3H each, s); EI-MS *m/z* 602 $[M]^+$, 533, 465, 341.

**ISOLATION OF HYDROXY CITRIC ACID**

**Isolation by alcohol precipitation method**

Finely powdered aqueous extract of dried fruits of *Garcinia indica* was dissolved in double distilled water. Pectins are removed by alcohol precipitation method and the filtrate was treated with 5 % potassium hydroxide solution and refluxed for three hours. Potassium salt of HCA was precipitated out and collected by subsequent filtration and evaporation to get salt of potassium hydroxy citrate[9].

**Extraction by using microwave energy**

Dried G. *indica* rind: After being washed and dried, the *G. indica* fruit was then cut to remove nuts and guts. The Garnicia rind was chopped, dried in the oven at 70-80 $^0$C for 36 hours until the rind was completely dried, powdered and then used for extracting organic acids.

After weighting about 10 g dried *G. indica* rinds, they were put into the distilled water in sample vessel 1000 mL and it was put into the microwave extraction system. The main factors selected for the extraction process were extraction time (A) Power (B) and materials ratio (C) (weight of the dried rinds: volume of the extracting agent). The optimum extraction conditions were determined by changing the parameters A, B, C. And then extracts was filtered through muslin cloth. This extracts was brown, opaque. The solution was treated with 4 g of activated charcoal and heated on a water bath for 30 min[10,11]. Then, proceeding to filter the extract with Buchner funnel and filter paper, activated charcoal was removed continuously by washing two times with 10 mL of distilled water for each time. The solution treated with activated charcoal became pale yellow. The extract was concentrated to 50 mL, and then treated with 100 mL of ethanol for 30 minutes to precipitate the remaining pectin (milky white precipitate substance). The floating solution and precipitate compounds were separated using the vacuum filter. After the precipitate part was washed 2 times with 20 mL ethanol for each time and filtered to collect the acid, the filtrate was made up to 100 mL by the mixing with the floating solution, followed by condensing and adding distilled water. The total acid amount in the sample was determined using acid-base titration method.

**Large scale isolation of Hydroxy citric acid**

Lewis and Neelakantan[12] isolated this (-)-HCA on a large scale from the dried rinds of *Garcinia*. The method consisted of extracting the acid by cooking the raw material with water under pressure (10 lb/in.2 for 15 min). The extract was concentrated, and pectin was removed by alcohol precipitation. The clear filtrate was neutralized with alkali, passed through cation exchange resin for recovery of the acid, which

was concentrated and dried. The crude dried mass was extracted with ether and recrystallized to give small needle shaped crystals of lactone.

Lewis[13] reported another method for the isolation of (-)-HCA from *Garcinia* using acetone. The acetone extract was concentrated and the acid taken up in water. The aqueous solution yielded the lactone on evaporation.

A process for large-scale isolation of HCA was demonstrated by Ibrahim and co-workers from the fresh/dried rinds of *Garcinia indica*. The dry rinds of the fruit were sliced and soaked in boiling water for about 20 h. The aqueous sodium hydroxide was added to the extract at around 80°C. Methanol was added to the extract until two layers are formed[14]. The lower layer contained the sodium salt of HCA, which was separated and neutralized by hydrochloric acid. Further, acetone was added to filtrate to obtain pure crystals of HCA.

**ISOLATION OF ANTHOCYANINS**

**By using liquid Nitrogen**

The plant material was frozen with **Liquid Nitrogen** and powdered using a Blender suitable for use under extremely low temperatures. The use of liquid nitrogen minimizes anthocyanin degradation by lowering the temperature and providing a nitrogen environment. The fine powder maximizes pigment recoveries due to its high surface area and favours disruption of cellular compartments. Later on from the crushed material anthocyanin was extracted by following Methanol and Acetone-chloroform extraction method. Later on the extract from respective methods was subjected to column chromatography and the absorbance was checked for fraction exhibiting pinkish colour (checked for all the fractions). TLC was also done for that fraction using particular mobile phase.[15,16]

**Isolation by using Hydrochloric acid and hydraulic press**

The fruits were washed, cut into four equal pieces (rinds) parallel to the major axis, then ground after the removal of seeds. The pulp thus obtained was mixed in a 1:2 ratio with acidified water (0.1% hydrochloric acid). The mixture was subjected to hydraulic press [17]. The extract was filtered using muslin cloth and stored in a cold room at 4–5 $^{0}$C and used for experiments as and when required.

**Purification of anthocyanins**

The aqueous extract of kokum was passed through a 500 mg sorbent C-18 Sep-Pak cartridge which was previously activated with methanol followed by 0.01% aqueous HCl. Anthocyanins were adsorbed onto the cartridge, whereas sugars, organic acids and other water soluble compounds were washed off the cartridge with 0.01% aqueous hydrochloric acid. Anthocyanins were eluted using acidified methanol (0.01%, v/v

HCl). Methanol was evaporated using a rotary evaporator at 35 $^0$C and pigments were dissolved in double distilled water containing 0.01% HCl. These partially purified concentrated anthocyanins were loaded (0.8 ml) onto a Sephadex LH20 column (1.0 x 60 cm) and eluted with a mixture of methanol/water/trifluoroacetic acid at a ratio of 20:79.5:0.5 [18]. Separated fractions from the Sephadex LH20 column were pooled and were concentrated using a lyophilizer separately.

## PHARMACEUTICAL IMPORTANCE OF KOKUM

### Anti-bacterial activity

Hexane and benzene extracts of the rinds of Kokum and its active constituent garcinol possess powerful anti-bacterial activity of its own. It also potentiated the effects of clarithromycin on H. pylori. Even the Kokum leaf extract possessed inhibitory activity against pathogenic bacteria Salmonella typhi, Salmonella parartyphi A and Salmonella tymphimurium. Aqueous extract of Kokam rind is reported to have highest antibacterial activity against Bacillus subtilis, followed by Escherichia coli, Enterobacter aerogenes and Staphylococcus aureus. The phytoconstituents garcinol, isogarcinol and xanthochymol exhibited inhibitory effect on the growth of methicillin resistant S. aureus[19].

### Anti-fungal activity:

Varalakshmi demonstrated antifungal activity of aqueous extract of Kokum rind against Candida albicans and Penicillium sp[19]. Chloroform extract of Kokum rind inhibited the growth of Aspergillus flavus and production of aflatoxin[5].

### Neuro-Protective activity:

Methanolic extract of Kokum fruit exhibited significant neuroprotective potential against 6-OHDA, indicating its anti-parkinson's activity in rats. Garcinol also reduced the expression of LPS-induced inflammatory mediators, iNOS and COX-2 and prevented nitric oxide accumulation in LPS-treated astrocytes[20]. It was also found to have anti-cholinesterase property. Cyanidin-3-glucoside prevent the neurite outgrowth and the expression of neurofilament proteins demonstrating its neuroprotective potential.

### Anti-neoplastic activity:

Garcinia indica fruit rind extract exhibited dose dependent cytotoxic activity by inhibiting cultured Balb/c 3T3 mouse fibroblasts. Previous reports showed that garcinol elicited inhibitory effect on Azoxymethane (AOM) - induced colonic aberrant crypt foci (ACF). Moreover, garcinol also improved liver glutathione-S-transferase and Quinone reductase levels, reflecting hastening of detoxification mechanisms. Garcinol showed significant suppression in 4-NQO induced oral carcinogenesis. It also diminishes tongue carcinoma. Garcinol prevented DNA damage, by scavenging the hydroxyl radical and inhibit

carcinogenesis. Furthermore, garcinol and its derivatives, cambogin, garcim-1, and garcim-2 showed potent growth-inhibitory effects on the neoplastic colon cancer cells, as well as in normal immortalized intestinal cells. Antiproliferative effects of garcinol was elicited in HeLa cells, human colorectal cancer cell line , human leukemia HL-60 cells, human breast cancer cells, prostrate and pancreatic cancer cells[21]. Isogarcinol and xanthochymol induce apoptosis through activation of caspase-3 in neoplastic cells. In vivo studies predicted reduction in number of non-malignant and malignant skin tumors per mouse in skin carcinogenesis model by Cyanidin-3-glucoside 18. Cyanidin-3-glucoside provided protection toCaco-2 colon cancer cells against the peroxyl radical (AAPH)-induced oxidative damage and reduce its cytotoxicity[22].

**Anti-inflammatory activity:**

Kokum rind aqueous and ethanolic extract was investigated for its anti-inflammatory potential, by using carrageenan induced paw edema model. Both the extracts showed powerful reduction in inflammation, in acute study. Moreover, significant reduction in lysosomal enzymes acid phosphate and alkaline phosphate confirms its anti-inflammatory activity [23].

**Cardio-protective activity:**

Cyanidin-3-glucoside of Kokum enhanced eNOS expression and increased the NO production, improving endothelial dysfunction, harmonizes blood pressure and may possibly prevent atherosclerosis[24].

**Anti-Obesity activity:**

The methanolic extract of the dried fruit of Kokum showed remarkable anti-hyperlipidemic activity in rats, using cholesterol induced hyperlipidemic model. Significant decrease in total cholesterol, triglycerides, LDL-C, VLDL-C levels and increase in HDL-C was reported by Darji[25]. Many studies have shown that intake of Hydroxycitric acid present in Kokam reduces appetite, inhibits lipogenesis and reduces body weight[26]. In vitro studies demonstrated increase in adipocytokine secretion and up-regulation of adipocyte specific gene expression without activation of PPARγ on treatment of rat adipocytes with cyanidin 3-glucoside. Furthermore, in vivo studies also showed increase in gene expression of adiponectin in the white adipose tissue[27]. Lipase inhibitory property and anti-obesity activity of isogarcinol was also shown.

**Traditional uses of Kokum**

Kokum is a favorite home-remedy useful as a:

- Digestive (Fruit)
- Antacid (Kokam Rind and leaves)
- Anti-dysentery (Fruit, Rind and leaves)

- Anti-diarrheal (Fruit, Rind and leaves)
- Anti-piles (Fruit, Rind and leaves)
- Anti-ulcer (Rind)
- Anti-colic (Rind and leaves)
- Anti-obesity (Fruit)
- Anthelmintic (Fruit)
- Anti-asthmatic (Fruit)
- Cardiotonic (Fruit)
- Hepatoprotective (Fruit)
- Anti-tumor (Fruit)
- Anti-hyperplasia (Leaves)
- Wound healer (Kokam butter)
- Analgesic (Rind, Fruit)
- Anti-inflammatory (Rind)
- Anti-dermatitis (Rind)
- Anti-perspirant (Rind)
- Astringent (Leaves, fruits and roots)
- Demulcent (Kokam butter)

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