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Research Article

Antimicrobial, Antioxidant Potential of *Garcinia Indica* by *Invitro* Method and Evaluation of Its Phytoconstituents

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Abstract

Garcinia indica (Kokum) trees are found in humid tropical regions of Western Ghats of India. Kokum is a tropical evergreen tree of moderate to large size. It grows to a height most useful part of the plant is the fruit of Kokum. This fruit is of commercial importance owing to its enormous medicinal properties. Kokum fruit is a popular condiment used in several states of India for making vegetarian and non-vegetarian "Curry" preparations, including the popular " Solkadhi".The rinds of ***Garcinia indica*** possesses an important phenolic compound called as Garcinol. There are many other compounds, beside garcinol which are present in the fruit and this study aims to investigate the antimicrobial properties of such compounds. Of all these compounds furfural and cyanidin-3-glucose are potent antimicrobials. The amount to which these compounds get extracted in different solvents determines the percent of bactericidal action. Extract of ***Garcinia indica*** was extracted by using various solvents like Ethanol, Methanol, Acetone and aqueous in Soxhlet Apparatus and antimicrobial test was tested by agar well diffusion method against various pathogens and Ethanol extract showed best results. Various concentrations of extract (10, 25, 50 and 100 µg/ml) were tested for in vitro antioxidant properties by reducing power ability test. Ferric reducing power of the extract was also evaluated by Oyaizu method Maximum antioxidant activity was observed at 100µg/ml. Ethanol and aqueous extract have shown the significant antimicrobial activity against *E.coli*, *Staphylococcus aureus, Micrococcus spp.,k.pneumoniae,Bacillus subtilis,Staphylococcus aureus,Pseudomonas aeruginosa* at 100µg/ml. Phytochemical investigation shows presence of active components such as tannins and glycosides .Conclusion: The results obtained from this study specify that fruit of ***Garcinia indica*** is a potential source of Antioxidants and thus could prevent many radical diseases and could be used as neutraceuticals.

Keywords: *Garcinia indica, Antimicrobial, Antioxidants, Phytochemical.*

Introduction

Garcinia indica (Kokum) trees are found in humid tropical regions of Western Ghats of India. 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 Kokan, Goa, North and South Kanara, North Malabar, Coorg and Wynad as well as in West Bengal and Assam. It is an androdioceious tree producing male and bisexual flowers on separate plants. It grows to a height most useful part of the plant is the fruit of Kokum. This fruit is of commercial importance owing to its enormous

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medicinal properties. Kokum fruit is a popular condiment used in several states of India for making vegetarian and non-vegetarian "Curry"preparations, including the popular "Solkadhi".(Parle Milind and Dhamija Isha *et al*., 2013)

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. The extract of the Garcinia indica has both antifungal (Selvi *et al*., 2003) and antibacterial properties and therefore, has a potential for use as bio preservative in food applications (Varalakshmi *et al*., 2010). Antimicrobial activity is primarily due to presence of furfural in Kokum extract. The anthocyanin present is also significant as antimicrobial agents. The prominent anthocyanin present is cyanidine-3-glucose (Sutar, R. L., Mane, S. P. and Ghosh, J.S., 2012). Its active constituent garcinol possess powerful anti-bacterial activity of its own.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reaction that may damage cells. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic. Hence use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the few decades. (Rajesh Kumar Rawri, K. Bharathi, K. N.Jayveera, SMB Asdaq, 2013)

Phytoconstituens are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties. Garcinia is a rich source of active compounds including garcinol, xanthochymol, isoxanthochymol and Hydroxycitric acid. These are flavonoids, benzophenones, xanthones, lactones and phenolic acids. The fruits contain citric acid, acetic acid, malic acid, ascorbic acid, hydroxycitric acid and garcinol. The major constituents of Kokum rind is garcinol, a polyisoprenylated benzophenones, isogarcinol and camboginol. Garcim-1, Garcim-2 and cambogin are chief oxidative products of garcinol,along with isogarcinol, gambogicacid mangostin, clusianone ,macurin, blongifolin (A,B,C), gutiferone (I,J,K,M,N).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.(Parle Milind *et al*., 2013)

Mechanism of Anthocyanin, Garcinol and Furfural

Anthocyanin has been shown to possess strong antioxidant activity. The two major anthocyanin pigments found in kokum are characterized by cynidine-3-gycoside and cynidine-3-sambubioside Anthocyanin's constitute approximately 2.4% of the total fruit biomass. There pigments can scavenge free radicals and are water soluble. Anthocyanins are group of important compounds which are part of flavonoids and responsible for red and purple color in fruits. Anthocyanins are based on a C-15 skeleton with a chromane ring having a second aromatic B-ring in position 2. The 3 and 4 -OH in B-ring determine radical scavenging capacity with a saturated 2, 3-double bond. Different glycosylation and hydroxylation positions determine their 12 potentials as an antioxidant. With increase in hydroxyl groups in B-ring, antioxidant activity increases when present as glucosides.

Garcinia indica fruit contains 1.5% of polyisoprenylated benzophenone derivatives called **Garcinol**. And contains phenolic hydroxyl groups. This makes it an

active antioxidant. It is also called a camboginol. It has β-diketone moiety and thus resembles a known antioxidant. Garcinol is a yellow colored, fat soluble pigment found in rinds of Kokum in 2-3%.

Furfural is an organic compound. Furfural dissolves readily in most polar organic solvents, but is only slightly soluble in either water or alkanes. Furfural, also called 2-furaldehyde, best known member of the furan family. It is a colorless liquid. It dissolves in water, and is completely miscible with alcohol and ether. The furfural formed is removed continuously with steam and concentrated by distillation.

Garcinia indica juice is a soothing drink in summer months and it provides relief from gastric disorder. It is traditionally used to treat sores, skin aliments such as rashes caused by allergies, dermatitis and chaffed skin, burns, scalds, and to relieve sunstroke. It is also a remedy for diarrhea, dysentery, piles and tumors. It facilitates digestion, purifies the blood and fights cholesterol. Kokum fruit contains rich amount of anti-oxidants that bind with free radicals and prevent oxidative damage to body cells. They also promote cell regeneration and repair.The hydroxycitric acid present in the Kokum fruit fights cholesterol and curbs lipogenesis and thereby, helps in weight loss. So considering all these the study was designed to investigate the antioxidant and antimicrobial potential of Garcinia indica. Phytochemical constituents are also investigated.

Material and Methods

Sample Collection

Amsul, the unsalted Kokum as well as salted Kokum are marketed. Lonavala kokum, Pakali Kokum, Khane or edible Kokum and Khoba Kokum are some of the trade varieties. Dried rinds of ***Garcinia indica*** fruit was collected from the local market of Sindhudurg district in Konkan region. Material was authenticated and kept for further use in refrigerator

Antimicrobial Activity of Extract of Dried Kokum (*Garcinia Indica*)

Rinds of ***Garcinia indica*** were cut into small pieces and dried into oven for 3hrs and coarse grinded into powder.10gm of *Garcinia indica* powder was weighed and filled into a foil paper and placed into a Soxhlet apparatus. By using various solvents like Ethanol, Methanol, Acetone and Aqueous the extract of ***Garcinia indica*** was extracted separately after 8hrs of extraction period.

Further the extract was concentrated up to 70ml and then stored into air tight container at 10^{o}C. Previously isolated organisms were taken from the microbiology laboratory of our college and preceded for their biochemical tests for confirmation. Organisms were confirmed and were further used to conduct antimicrobial assay by using ***Garcinia indica.*** Antimicrobial test were performed by well diffusion method in triplicates, incubated for 24hrs and zone of inhibition were measured and recorded.

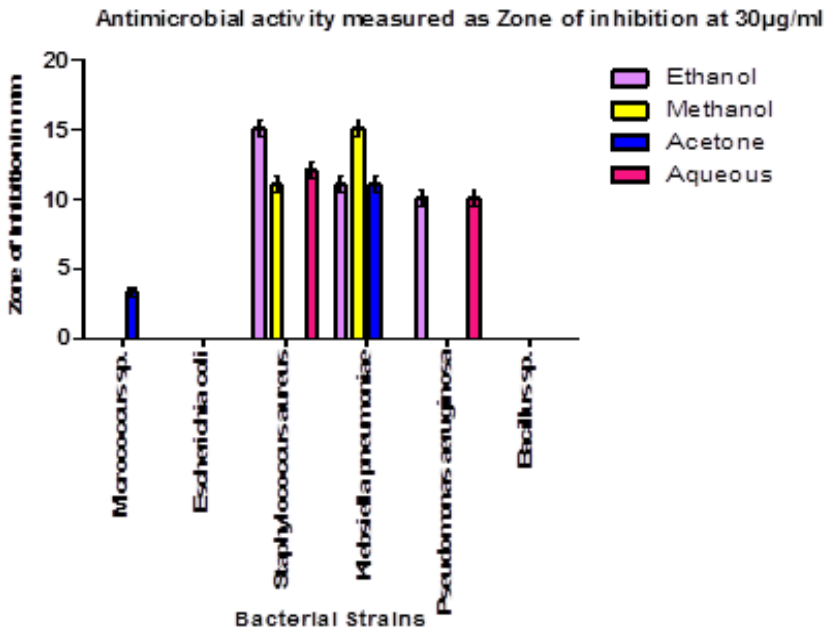
Reducing Power Ability (Oyaizu Method)

The ferric reducing capacity of extracts was investigated by using the potassium ferrocyanide-ferric chloride method (Khan, P. *et al*., 2011). Briefly, 0.2 mL of each of the extracts at different concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferrocyanide $K_3Fe(CN)_6$ (1%) were mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 1000 rpm for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance against the corresponding sample concentration. Ascorbic acid was used as a reference compound.

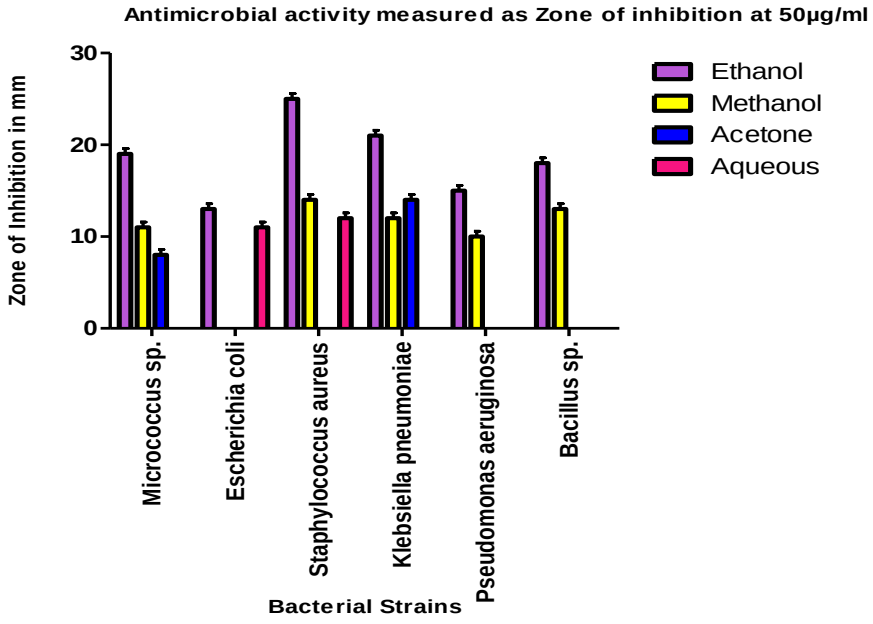
Evaluation of Phytochemical Screening

Preliminary Evaluation of phytochemical screening as (Nagendra, K., R, Kusum.and H, D. Ramachandran.,2014) Qualitative phytochemical analysis of crude extract determines the presence of compounds like sterols, glycosides, saponins, tannins, flavonoids, phenols, resins, and alkaloids using the standard protocol. The qualitative phytochemical screening of crude extracts was done using the methods described by earlier researchers.

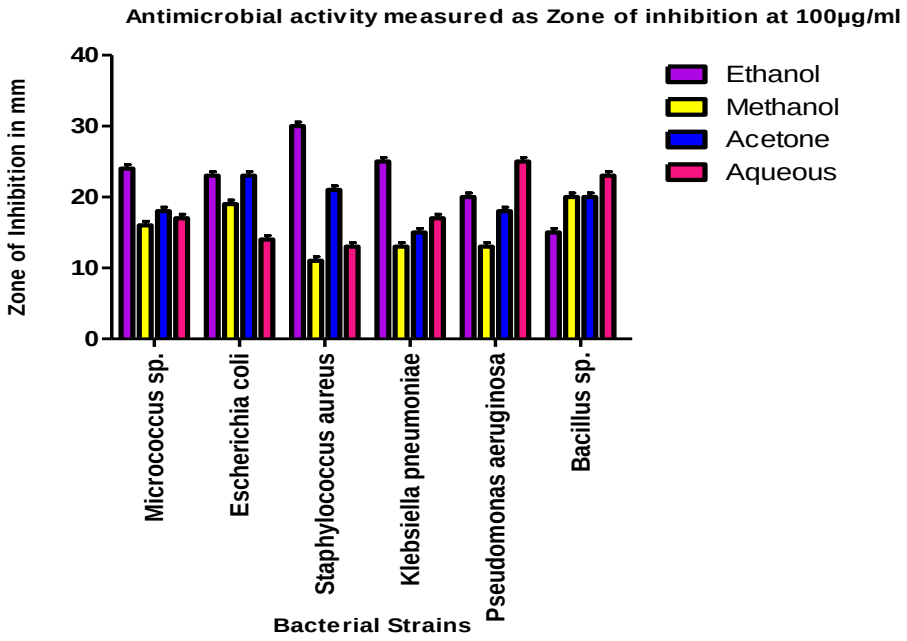
Results



A) Antimicrobial Activity of Different Extracts of Garcinia Indica (30µg/ml)



B) Antimicrobial Activity of Different Extracts of Garcinia Indica (50µg/ml)



C) Antimicrobial Activity of Different Extracts of Garcinia Indica (100µg/ml)

Table No: 1- Reducing Power Assay

	Dose(µg/ml)	**Reducing power(700nm)**
Vitamin C (Ascorbic Acid)	100	1.91
	50	1.88
Garcinia indica	100	1.73
	50	1.54
	25	1.47
	10	1.11

Table No: 2- Phytochemical Screening

Sr No.	**Test for**	**Results**
1	Carbohydrates	**+**
2	Resins	**-**
3	Tannins	**+**
4	Glycosides	**+**
5	Flavonoids	**-**
6	Saponins	**-**

Discussion and Conclusion

By referring the Bergey's Mannual of Determinative Bacteriology and performing the biochemical tests the organisms were confirmed up to the Genus and species level are Escherichia coli, Bacillus sp., Klebsiella pneumonia, Micrococcus sp., Pseudomonas aeruginosa, and Staphylococcus aureus.

Antimicrobial Assay

From the graphical analysis it has been concluded that ***Staphylococcus aureus*** in presence of ethanol extract shows 15mm zone of inhibition at 30µg/ml volume, 25mm zone of inhibition at 50µg/ml volume and 30mm zone of inhibition at 100µg/ml volume respectively. ***Klebsiella pneumoniae*** also shows highest 15mm zone of inhibition at 30µg/ml volume in presence of ethanol extract. Kokum showed antibacterial activity against Gram positive and Gram negative organisms.

Escherichia coli was not inhibited by methanol extract by 100µg/ml volume (Sutar, R. L., Mane, S. P. Ghosh, J.S, 2012).Results showed that ***Escherichia coli*** was inhibited by all the extracts at 100µg/ml volume. Acetone extract of kokum fails to inhibit the Gram negative organisms (Sutar, R. L., Mane, S. P. Ghosh, J.S, 2012).Acetone extract of kokum inhibits Gram negative organisms at higher concentration of 100µg/ml, whereas ***Klebsiella pneumoniae*** was inhibited at lower volume too. ***Escherichia coli*** is not inhibited by any extracts except the ethanol extract of 30µg/ml volume (Sutar, R. L., Mane, S. P. Ghosh, J.S, 2012).It was observed that ***Escherichia coli*** was not inhibited by all the extracts at the concentration of 30µg/ml. Antimicrobial activity of all kokum extracts against Gram positive organisms increases as the volume of extract increases. (Sutar, R. L., Mane, S. P. Ghosh, J.S, 2012) According to results it was found that Antimicrobial activity of all kokum extract against both Gram negative and Gram positive organisms" increases as the volume increases. Ethanol and water extract showed significant zone of inhibition against Gram negative organisms (Sutar, R. L., Mane, S. P. Ghosh, J.S, 2012) According to the observation more significant results was shown by only ethanol extract against both Gram negative and Gram positive organisms.

Antioxidant Activity

The reducing power assay of ethanol extract of ***Garcinia indica*** was found to be correlated with increasing concentrations (at 700nm) as compared with Ascorbic acid, which is a known antioxidant. The presence of reductones are responsible for reducing capacity, which involved in prevention of chain inhibition of metal ions, decomposition of peroxides and radical scavenging.(Rajesh Kumar Rawri *et al.*,2013). Higher the absorbance indicates Higher reducing Power. In conclusion, ethanol extract of ***Garcinia indica*** showed dose dependent antioxidant properties in in vitro evaluation.

Phytochemical Screening

The fruit of ***Garcinia indica*** were tested with respect to their phytochemical constituents and antioxidant activity by reducing power assay. Extraction was performed using soxhlet apparatus and aqueous as a solvent. The results indicated that the examined fruit of ***Garcinia indica*** contains certain amounts of active components (phytochemical constituents), proving them to be perfect sources of antioxidants.

Conclusion

The extract of Kokum exhibited higher reducing activity indicating fruit extract of Garcinia indica as a prospective candidate for antioxidant study for further studies. Also isolation, purification, and characterization of the phytochemicals will make significant studies. This primary information will simplify in leading further studies on the discovery of bioactive ingredients, resolve their efficacy by in vivo studies, and demonstrate their safety and effectiveness in clinical trials. The study suggests that crude extract possesses promising antimicrobial and antioxidant activity and antimicrobial activity. So medicines can be generated from these for possible therapeutic applications. This is the fundamental collective report on antimicrobial, antioxidant activity of Garcinia indica and qualitative analysis of its phytochemical constituents.

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