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Comparison between the Traditional and Lab Scale Extraction of Kokum Oil and Determination of the Effect of Different Solvent on Yield of Extract and Study It's Antibacterial and Antifungal Activity

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ABSTRACT

This study focused on the extraction of oil from kokum (Garcinia indica) seeds and the evaluation of its antimicrobial properties. Oil was extracted using a laboratory-scale Soxhlet apparatus with acetone, chloroform, and ethanol, and also by a traditional boiling method. The objectives were to compare the percentage yield and antimicrobial efficacy of the different extracts. Phytochemical screening confirmed the presence of phenolic compounds as, and Thin Layer Chromatography (TLC) suggested the presence of garcinol. Results showed that Soxhlet extraction provided a higher yield than the traditional method, with acetone giving the highest yield (87.5%). Antimicrobial tests were conducted against Staphylococcus aureus, Pseudomonas aeruginosa, and Aspergillus niger. Despite having the lowest yield (35.75%), the chloroform extract exhibited the strongest antibacterial and antifungal activity, while the traditional extract showed the least.

Keywords: kokum, kokum butter, Garcinol, Clusiaceae, Soxhlet extraction ,Traditional boiling method, Antimicrobial activity, Zone of inhibition, Agar well diffusion method, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Phenolic compounds, Thin Layer Chromatography

INTRODUCTION

Kokum butter, a type of fat found in kokum seeds, is beneficial. It is employed in the confectionery and chocolate industries. It is occasionally employed in the ointment and surfactant industries as well. Kokum butter is regarded as emollient, demulcent, astringent, and nutritious. It works well for pharmaceutical applications, ointments, and suppositories. It is applied locally to hand, lip, and other fissures and ulcers. After oil is extracted, the oil cake that remains can be used to make industrial adhesives, manure, and animal feed. the various kokum fruit sections.[1]

At normal temperature, the 23%–26% oil in Garcinia indica seeds stays solid. Cosmetics, medications, and disinfectants are all made with it. The fruit's seeds produce 23–26% by weight of seeds and roughly 44% by weight of kernels, a desirable edible fat that is marketed as "Kokum butter" It is primarily extracted on a cottage industry basis by either churning the crushed pulp with water or by crushing the kernels, boiling the pulp in water, and then skimming the fat off the top.[1]

The confectionery, pharmaceutical, and cosmetic industries all have a strong need for kokum butter, which is made from the seeds. When manufacturing chocolate, kokum butter is used instead of cocoa butter since it has similar solidification properties, tolerance to milk fat, and compositions of fatty acids and triacylglycerols. Furthermore, research has demonstrated that adding kokum butter to cocoa butter improves the chocolates and cocoa butters ability to withstand heat, preventing heat-induced softening and consistency loss. The use of kokum butter helps treat phthisis pulmonalis, diarrhea, dysentery, and scorbutic disorder. Kokum butter has been shown to have wound-healing qualities and to be helpful in treating inflammatory sores, ulcerations, lip and hand fissures, and chapped skin. Candles and soaps are also made from kokum butter. The seed butter is used to treat mucous diarrhea and dysentery. The root, bark, fruit, and seed oil is used to cure worm infestations, piles, stomach disorders, and mouth diseases. It can be applied topically to allergic rashes and other skin disorders, or it can be infused. Kokum butter is an emollient that can be used to treat burns, scalds, and chaffed skin.[2]

Kokum seeds contain high levels of glycerides of stearic acid (55%) oleic acid (40%), palmitic acid (3%), linoleic acid (1.5%), hydroxyl capric acid (10%), and meristic acid (0.5%). About a quarter of kokum seed is edible fat, commonly known as kokum butter. It is typically extracted by spinning the seeds in water or solvent extraction, or by smashing the seeds and boil the seed in hot

water, and then fat removed from top. The yellowish crude kokum butter serve as an edible fat or can be used as an adulterant in ghee. White-colored refined Kokum butter is equivalent to high-quality hydrogenated fats. As much as 7.2% of the total consumption of kokum butter consist of free fatty acids. It is utilized in the cosmetics sector to create soaps, lotions, creams, and lip balms.[2 3 4]

Kokum butter is one of the most stable unusual butters that doesn't need to be refrigerated due to its comparatively high melting point. The existence of chemical compounds and their importance in preventing illness are supported by numerous studies.[2]

The oil found in kokum seed kernels, which is marketed as "kokum butter," ranges from 33 to 44 percent.[5 6] Kokum fruit contains 1.5% of polyisoprenylated benzophenone derivative called Garcinol. Garcinol is a yellow coloured, fat soluble pigment found in the rinds of Kokum at level of 2-3%. In fact, all Garcinia species have some amount of garcinol.[5]Garcinol is a phenolic compound from Garcinia indica fruit rinds that has demonstrated antibacterial activity against the range of bacterial species. It works as antibacterial agent by inhibiting key enzyme like topoisomerases.[7 8]

Antimicrobial Activity

Antimicrobial activity is defined as the ability of probiotic bacteria to inhibit the growth of pathogenic bacteria by producing inhibitory substances, such as organic acids, hydrogen peroxide, and bacteriocins, which exert antagonistic effects on pathogens.[9]

The diffusion method of agar wells was used. Both Gram positive and Gram negative bacteria were grown on nutrient agar. The appropriate organism was seeded into the cooled molten media. Following the medium's solidification, 5 mm and 8 mm wells were made and filled with 30 µl, 50 µl, and 100 µl of extract made with various solvents as well as controls made entirely of solvent. Three duplicates of each test were conducted. For twenty-four hours, the plates were incubated at 25°C. After deducting that from the corresponding pure solvent, the zone of inhibition was measured and noted.[110]

1. Antibacterial Activity

Antibacterial activity refers to all active principles that inhibit bacterial growth, prevent microbial colonies from forming, and may kill microorganisms.Kokum rind extract containing hexane, benzene and garcinol exhibits potent anti-bacterial activity.[11]

The Agar Well Diffusion method was employed to evaluate the antibacterial properties of G. indica against various bacterial species, including Staphylococcus aureus and Pseudomonas aeruginosa, Escherichia coli, Micrococcus luteus, and Micrococcus aureus. Fruit from G. indica was extracted using water, methanol, ethanol, and acetone at concentrations of 30 µl, 50 µl, and 100 µl per extract.[1 12 13]

2. Antifungal activity

The most contagious plants are pathogenic fungi, which alter growth phases and post-harvest development.Numerous fungal species found in fruits and vegetables can result in genetic issues with the factor, the quanti ty of nutritious food, the organoleptic qualities, and the short shelf life.Additionally, the fungus can occasionally produce mycotox ins or allergens that indirectly trigger allergic reactions or sensitivities in consumers.Some researches have looked at the kokum ri nd aqueous extract's antifungal efficacy against penicillium and Candida albicans.Kokum rind extracts in chloroform inhibit the gr owth of Aspergillus flavus and the generation of aflatoxin.[11 13]

Plant Profile[1 14]

Fig.1 kokum fruits

Common Name : Kokom

Biological Name : Garcinia Indica

Family : Clusiaceae

Division : Magnoliophyta

Subfamily : Garcinieae

Genus : Garcinieae

Class : Magnoliopsida

Subclass : Dilleniidae

Species : Garcinia Indica choisy

Biological source : Kokum butter is the fat obtained by expression from the seeds of garcinia indica plant.

Plant description : Garcinia indica is a medium-sized evergreen tree. It grows to a height of about 18 m. The tree has drooping branches.

The berries ripen in the summer. They are spherical with a diameter of about 5 cm. They have indentations on the top, on the stalk, and on the bottom. Each berry has 5 to 8 seeds surrounded by a sweet and sour pulp that contains some fibres. They are initially green, but turn red as they ripen.

LITERATURE REVIEW

Maurya.et.al.,(2023) The aim of this review article provides information mainly on various pharmacological activities like anti-bacterial, anti-helminthic, anti-inflammatory, antacid, anti-ulcer, cardio protective, UV protection, anti-hyperglycemic, protective effect against Parkinson disease, treatment for newly acquired or recently active traumatic disease, anti-cancer, anti-hyaluronidase and elastase, anti-obesity, anti-arthritic. Oil is extracted from kokum seeds. Kokum butter, a form of oil, is commonly utilized in curries, cosmetics, pharmaceuticals, and luxury confectionery recipes across different nations. Garcinia indica fruit extract has the potential to be utilized for biogenic generation of AgNPs with antibacterial and antioxidant properties that might be utilized in commercial biomedical applications.

SB Kalse.et.al.,(May 20, 2024) This review article examines the different extraction methods of kokum butter, a natural plant-based fat obtained from the kokum seeds. The traditional methods include sun drying, boiling, and hand-milling, while modern techniques include solvent extraction, supercritical fluid extraction, and enzymatic extraction. Each method has its advantages and limitations in terms of yield, purity, and environmental impact.

Shrikant Baslingappa Swami.et.al., (2014) This review aimed to provide a summary of the functional, medicinal, and physiological characteristics of the kokum fruit. Kokum (Garcinia Indica Choisy) is a traditional fruit that is commonly enjoyed as sarbat. Kokum is a fruit tree utilized for culinary, pharmaceutical, nutraceutical, and industrial purposes. Kokum has a rich history in Ayurveda medicine, as it was traditionally employed to address sores, dermatitis, diarrhea, dysentery, ear infections, and to aid digestion. Kokum seeds are utilized for extracting oil. The oil is known as kokum butter and is utilized in curries, cosmetics, medicines, and expensive candy recipes abroad. The kokum fruit serves as an antioxidant, acidulant, and appetite booster while aiding in the battle against cancer, paralysis, and cholesterol.

Rahul C Ranveer.et.al., (October 12, 2017) The main focus of the review is to explore bioactive constituents of Kokum (Garcinia indica Choisy) and their health benefits. Kokum generally grown in tropical region and kokum has many bioactive compounds such as anthocyanin, Hydroxyl citric acid and Garcinol, those possesses several nutraceutical activities. Among the bioactive compound anthocyanin, this has a great potential as a natural colorant and having free radical scavenging activity. The hydroxyl citric acid (HCA) is major acid present in kokum, which is used as an anti-obesity ingredient in pharmaceutical preparations.

R S Bhande.et.al., (June 2017) The primary emphasis of research on this Kokum butter is seen as nourishing, calming, tightening, and hydrating. Petroleum ether and chloroform served as solvents for extracting oil from Kokum seeds at a fixed extraction

temperature and residence time with different particle sizes.. Of the two solvents, Chloroform produced the highest oil yield from Kokum seed. The selection of solvent and particle size influenced the oil yield obtained using the Soxhlet Apparatus. This research highlights the potential medicinal applications of Kokum seed oil, alongside its established use in cosmetics in India.

Sutar R. L.et.al., (2012) The study main focus on the kokum fruit is mainly found during the summer months, and the rind of the fruit is salted and dried and preserved for use in other parts of the year when it is not available fresh. The rind contains a significant phenolic compound known as Garcinol. The significance of this compound is widely recognized. This study, consequently, is not intended to examine this compound. In addition to garcinol, various other compounds are found in the fruit, and this research seeks to examine the antimicrobial effects of these compounds. The substances include furfural and its derivatives, cyanidin-3-glucose found as anthocyanin in the peel, along with caffeine. Among these, furfural and cyanidin-3-glucose are strong antimicrobials. The degree to which these compounds are extracted in various solvents influences the level of bactericidal activity.

Dr. Sherin Justin et.al., (2018) The study mainly focused on the in-vitro antibacterial activity of the kokum butter. The ethanolic and aqueous extracts of Kokum were prepared by soxhlation and maceration methods respectively. The screening of the extracts for their antibacterial activity was done by disc diffusion method. Minimum inhibitory concentration of the extracts was also determined by agar dilution method.

S. P. Dehankar et.al., (2018) The study mainly focused on the Kokum butter is an extract made from the Garcinia indica Choisy seeds. Astringent, nutritious, emollient, and demulcent are some of the beneficial qualities of this kokum butter. Oil was extracted using acetone, ethanol, and chloroform as solvents. After the analytical testing, it was discovered that utilizing acetone produced a higher-quality product. In addition to the oil's popularity as a cosmetic in India, this comparative investigation of solvents to extract butter from kokum seeds offers prospects for the oil's medical usage**.**

MATERIALS AND METHODS

Preparation of crude drug powder [15–20]

1) Collection of seed: Kokum fruits were gathered from the jungle in the Ratnagiri district of Maharashtra. After slicing open the fruits, the seeds were extracted from the pulp and given a thorough water wash.

2) Washing of seed: The seeds were surface washed with distilled water three to four times for cleaning and then dried.

3) Drying of seed: Seed are dried by sun drying after that the seed are dried in Hot air oven for 130 °C for 1 hour to remove the remaining moisture.

4) Crushing and Sieving: dried seed are crushed in the mixer and passed from sieve no.21.

Extraction of oil

- **Procedure**: The laboratory scale soxhlet apparatus was used to extract the oil from seed. The dried seed powder is mixed with the solvent (Acetone, ethanol, Chloroform) in the ratio of 1:10 and the mixture is transfer to the soxhlet extractor. Adjust the temperature of heating mantle equal to the boiling point of solvent. Separate the solvent from the oil extract by evaporating the solvent.

Fig. 2 Experimental Setup of Soxhlet Extractor

1) **Extraction of oil by using Acetone**

- Take 20 gram of the kokum seed powder.
- Dissolved it to 200 ml Acetone (ratio of 1:10).
- Place the above mixture in the Round Bottom flask. Attached the flask to the soxhlet apparatus.
- Adjust the temperature of heating mantle to 56°C (Boiling point of Acetone).
- Remove the solvent mixture after the completion of 12 cycle of extraction.
- For separation of oil extract from solvent evaporate the solvent on hot plate.
- Measure the yield of extract.

Extraction of oil by using chloroform

- Take 20 gram of the kokum seed powder.
- Dissolved it to 200 ml chloroform (ratio of 1:10).
- Place the above mixture in the Round Bottom flask. Attached the flask to the soxhlet apparatus.
- Adjust the temperature of heating mantle to 61°C (Boiling point of Acetone).
- Remove the solvent mixture after the completion of 12 cycle of extraction.
- For separation of oil extract from solvent evaporate the solvent on hot plate.
- Measure the yield of extract.

2) **Extraction of oil by using solvent Ethanol**

- Take 20 gram of the kokum seed powder.
- Dissolved it to 200 ml Ethanol (ratio of 1:10).
- Place the above mixture in the Round Bottom flask. Attached the flask to the soxhlet apparatus.
- Adjust the temperature of heating mantle to 78.37°C (Boiling point of Acetone).
- Remove the solvent mixture after the completion of 12 cycle of extraction.
- For separation of oil extract from solvent evaporate the solvent on hot plate.
- Measure the yield of extract.

Fig.3 acetone extract

Fig. 4 chloroform extract

Fig. 5 Ethanol extract

Extraction of oil by Traditional Method [23]

- Firstly kokum seed are collected and crushed.
- The powder of seed is mix with water and then the water is boiled up to the oil is floats on the boiling water.
- After separating the oil layer from water it becomes solidified after cooling.
- Separate the butter from water.

Fig. 6 Boiling of seed powder with water

Fig.7 kokum extract by traditional method

PRILIMINARY PHYTOCHEMICAL SCREENING [24]

Test for carbohydrates

Test	Procedure
Molisch's test	To 2-3 ml of the aqueous extract, mix in a few drops of alpha-naphthol solution in alcohol, shake well, and then add concentrated solution. H_2SO_4 on the walls of the test tube. A violet ring forms at the interface of two liquids
Fehling's test	Combine 1 ml of Fehling's A with 1 ml of Fehling's B solutions, then heat to boiling for one minute. Incorporate an identical amount of test solution. Warm in a boiling water bath for 5-10 minutes. Initially, yellow is seen, followed by brick red precipitation.

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Test for proteins

Test	Procedure
Biuret test (General test)	To 3 ml T.S. add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears.
Million's test	Mix 3 ml T.S. with 5 ml million's reagent, white ppt. appears warm ppt. turns brick red or the ppt. dissolves giving red coloured solution.

Test for phenolic compound

Test	Procedure
5% FeCl3 solution	Add 5% FeCl3 solution to extract gives Deep blue colour
Lead acetate	Add Lead acetate to extract gives white ppt.
Gelatin solution	Add Gelatin solution to extract gives white ppt.
Acetic acid solution	Add Acetic acid solution to extract gives Red colour solution.

Test for flavonoids

Test	Procedure
Shinoda Test	To dry powder or extract, add 5 ml 95% ethanol/t-butyl alcohol, few drops conc. HCI and 0.5 g magnesium turnings. Orange, pink, red to purple colour appears (flavonols, dihydro derivatives and xanthene's). Add t-butyl alcohol before adding the acid to avoid accidents from a violent reaction an to dissolve the coloured compounds into the upper phase. By using zinc instead magnesium, only flavanols give a deep red to magenta colour while flavanones an flavonols give weak pink to magnetic colours or no colour.
Sulphuric Acid Test	When sulphuric acid (66% or 80%) is added, flavones and flavono dissolve, producing a deep yellow solution, while chalcones and narones yield red or reddish-blue solutions. Flavanes provide colours from orange to red.
Lead acetate solution	To small quantity of reside, add lead acetate solution Yellow coloured precipitate formed

Test for alkaloids

Test	Procedure
Dragendorff's test	Add a few drops of Dragendorff's reagent to 2-3 ml of the filtrate. An orange-brown precipitate will form.
Wagner's test	2-3 ml filtrate with few drops Wagner's agent gives reddish brown ppt.

5.5 Chromatographic estimation of Garcinia Indica[25]

Method: Thin layer chromatography

Sample: Kokum oil

Stationary phase: Silica gel plate

Mobile phase: Toluene: Ethyl acetate: Formic acid (7:3:0.5)

Detection: Under UV light (254nm)

Procedure

1**. Preparation of Sample**: Take a Kokum oil

2. **Preparation of mobile phase**: Make a mobile phase of solvent toluene: ethyl acetate: formic acid in ratio of 7:3:0.5. Close the mobile phase chamber with Aluminium foil.

3. **Preparation of TLC plate**: place the sample on TLC plate with the help of capillary and let it dry and repeat the procedure 3-4 times.

4. **Develop the TLC plate**: Place the prepare TLC plate into the chamber and allow the mobile phase to run on stationary phase.

5. **Detection**: After maximum running of mobile phase remove the TLC from chamber and allow it to dry and detect the spot under UV light (254nm).

Antimicrobial Activity [26 27]

The in-vitro antimicrobial activity of kokum butter was evaluated by agar well diffusion method (cup plate method). This method was employed as it is simple, inexpensive, rapid result and suitable for various microorganism. The microorganism used for this included Bacteria like Staphylococcus aureus and Pseudomonas aeruginosa[12]and fungi like Aspergillus niger.

Procedure[110]

1. **Sterilization of glassware**: Petri plate are wash under the tap water then clean with the alcohol swap. Wrap with paper and place in the hot air oven for specific period of time. All glassware are also washed and placed in oven for sterilization.

2. **Preparation of media**: Different media are prepared for bacteria and fungi.

- Bacterial media: Agar media is prepared by weighing required amount nutrient agar and mixed with water, by gently providing heat to medium for efficient dissolution of agar in water. The necessary quantity of agar and water determined based on the standard provide (28g in 1000ml). [28]

- Fungal media: Agar media is prepared by weighing required amount of Sabouraud dextrose agar and mixed with water, by gently providing heat to medium for efficient dissolution of agar in water. The necessary quantity of agar and water determined based on the standard provide (65g in 1000ml).[29 30]

3. **Sterilization of media**: Both the media are sterilize by placing in the autoclave for 20 min.

4. **Preparation of Petri plate**: After the sterilization of media the media is pour into the Petri plate at equal manner.

5. **Inoculation of microorganism**: both microorganism (bacteria and fungi) are introduce into the media by spread plate method (by spreading the bacteria on the media with the help of spreader).

6. **Wells or cups in the agar by using sterile crock borer were created**.

7. **Addition of test substance**: the test substance is introduce in to the wells or cups with the help of the micropipette with different concentration. Also add control solution in other Petri plate to check ZOI of solvent.

8. **Incubation** : The bacterial and the fungi species are incubated for different time interval.

- Bacterial species: Incubated for 24hr-48hr in 37° C into the incubator.

- Fungi species: Incubated for 7 days in 37° C in to the incubator.

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9. **Zone of inhibition measure by measuring the diameter of clear area around the well**.

Fig.8 Prepared culture media

Fig.9 Preparation of Petri plate's

RESULTS AND DISCUSSION

Yield of extract

Table no. 1 Yield of extraction

Type of extraction		%Yield of Extract
1. Soxhlet Extraction	SOLVENT USED	
	1) Acetone	87.5%(7gm)
	2) chloroform	35.75%(2.86gm)
	3) Ethanol	68.75% (5.5gm)
2. Extraction by Traditional method		46.25% (3.7gm)

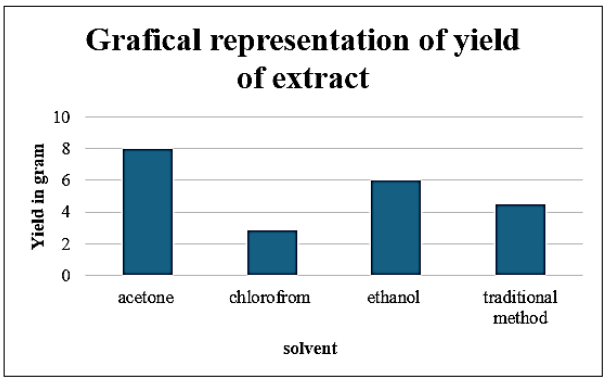

Fig.10 effect of different solvent on yield of extract

The kokum kernel contain the 40% fat of their total weight. According to the study the laboratory scale soxhlet extraction gives the higher yield of extract than the traditional method in laboratory scale soxhlet extraction the yield of extract depend on the solvent use for the extraction. Comparing between Acetone, Ethanol and Chloroform the acetone gives the higher yield of extraction and the chloroform gives the lower yield of extraction. Yield obtain from chloroform is lower than the traditional method.

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Preliminary phytochemical screening

Table no. 2 Preliminary phytochemical screening

Phytochemical	Test	Observation	Inference
Test for carbohydrates	Molisch's test	No Violet ring is formed at the junction of two liquids	Carbohydrates are absent
	Fehling's test	No brick red ppt. is observed	Carbohydrates are absent
Test for proteins	Biuret test	No Violet or pink colour appears	Protein is absent
	Million's test	No red ppt.	Protein is absent
Test for phenolic compound	5% FeCl3 solution	Deep blue colour	Phenolic compound is present
	Lead acetate solution	white ppt.	Phenolic compound is present
	Gelatin solution	white ppt.	Phenolic compound is present
	Acetic acid solution	Red colour solution	Phenolic compound is present
Test for Flavonoids	Shinoda Test	No pink colour shows	Flavonoid is absent
	Sulphuric Acid Test	No orange or red colour	Flavonoid is absent
	Lead acetate solution	No yellow colour ppt.	Flavonoid is absent
Test for Alkaloids	Dragendorff's test	No Orange brown ppt.	Alkaloids is absent
	Wagner's test	No reddish brown ppt.	Alkaloids is absent

The phytochemical screening of kokum oil gives the positive test for Phenolic Compound. Kokum oil contain garcinol which belong to chemical class phenolic compound (polyisoprenylated benzophenone). So the positive test for phenolic compound indicates the presence of garcinol.

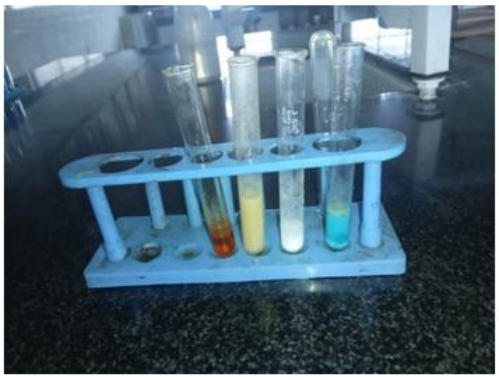

Fig.11 Test for phenolic compound (soxhlet extraction)

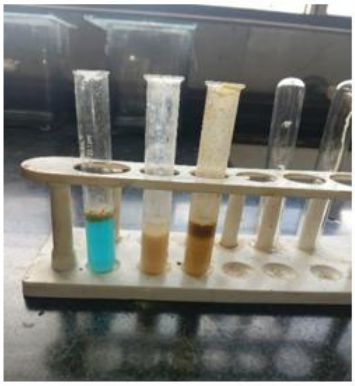

Fig.12 Test for phenolic compound (traditional method)

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Chromatographic estimation of Garcinia Indica

Fig.13 TLC of kokum oil (soxhlet extract)

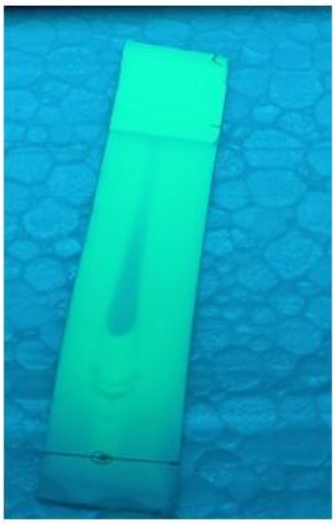

Fig.14 TLC of kokum oil (traditional method)

The benzophenone shows the blue fluorescent band under the UV light (254nm). Where the garcinol is the polyisoprenylated benzophenone which confirms the presence of garcinol in kokum oil. The mixture of Toluene, Ethyl acetate and Formic acid (in ratio of 7:3:0.5) was utilized, and plate where observed under UV light gives the blue fluorescent band.

Antimicrobial Activity

The Zones obtained by antibacterial action of kokum seed extract are enlisted in below tables.

A. Antibacterial activity

1. Control solvent

Table no. 3 Zone of inhibition obtain from control Solvents

Fungal species	**Acetone**	**Chloroform**	**Ethanol**
	Concentration of solvent		
Staphylococcus aureus	**30µg/ml**	**30µg/ml**	**30µg/ml**
	Zone of inhibition		
	-	4mm	6 mm

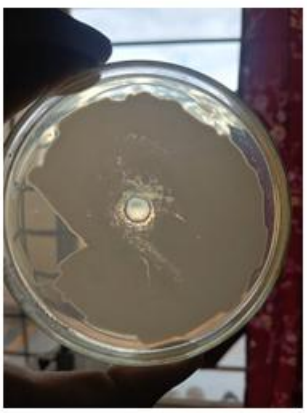

Fig.15 Control (ethanol)

Fig.16 Control (acetone)

Fig.17 Control (chloroform)

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2. Acetone Extract

Table no. 4 Zone of inhibition obtain from Acetone extract

Bacterial species	Concentration of extract			
	30 µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition in mm			
Staphylococcus aureus	13mm	14mm	16mm	18mm
Pseudomonas aeruginosa	12mm	14mm	15mm	17mm

Fig.18 S. aureus (Acetone)

Fig.19 P. aeruginosa (Acetone)

3. Chloroform extract

Table no. 5 Zone of inhibition obtain from Chloroform extract

Bacterial species	Concentration of extract			
	30 µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition in mm			
Staphylococcus aureus	17mm	18mm	19mm	22mm
Pseudomonas aeruginosa	15mm	18mm	19mm	20mm

Fig.20 S. aureus (chloroform)

fig.21 P. aeruginosa(chloroform)

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Table no. 6 Zone of inhibition obtain from Ethanol extract

Bacterial species	Concentration of extract			
	30 µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition in mm			
Staphylococcus aureus	12mm	15 mm	16mm	18mm
Pseudomonas aeruginosa	10mm	12mm	15mm	20mm

Fig.22 S. aureus (Ethanol)

Fig.23 P. aeruginosa (Ethanol)

5. Extract by traditional method

Table no. 7 Zone of inhibition obtain from Extract acquired from traditional method

Bacterial species	Concentration of extract			
	30µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition			
Staphylococcus aureus	7mm	8 mm	10mm	13mm
Pseudomonas aeruginosa	6mm	9mm	11mm	15mm

Fig.24 S. aureus

fig.25 P. aeruginosa

According to antibacterial research, extracts obtained via laboratory Soxhlet extraction demonstrate superior activity, resulting in a larger zone of inhibition compared to the traditional method. Comparing between Acetone, Ethanol and Chloroform extract the

chloroform extract gives greater zone of inhibition for S.aureus (22mm) and for P aeruginosa (20mm). Ethanol extract gives the slightly greater zone of inhibition for S.aureus (18mm) and for P aeruginosa (20mm) then acetone which gives for S.aureus (18mm) and for P aeruginosa (17mm). As the concentaion of oil increases the zone of inhibition increases.

B. Antifungal activity

1. Control solvent

Table no. 8 Zone of inhibition obtain from control Solvents

Fungal species	Acetone	Chloroform	Ethanol
	Concentration of solvent		
aspergillus niger	30µg/ml	30µg/ml	30µg/ml
	Zone of inhibition		
	-	-	4mm

Fig .26 Control (Acetone)

Fig.27 Control (Chloroform)

Fig. 28 Control (Ethanol)

2. Acetone extract

Table no. 9 Zone of inhibition obtain from Acetone extract

Fungal species	Concentration of extract			
	30µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition			
aspergillus niger	6mm	8mm	9mm	10mm

3. Chloroform extract

Table no. 10 Zone of inhibition obtain from Chloroform extract

Fungal species	Concentration of extract			
	30µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition			
aspergillus niger	15mm	20mm	22mm	25mm

4. Ethanol extract

Table no. 11 Zone of inhibition obtain from Ethanol extract

Fungal species	Concentration of extract			
	30µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition			
aspergillus niger	11mm	13mm	15mm	20mm

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Fig. 29 A. niger (acetone)

Fig. 30 A. niger (Chloroform)

Fig. 31 A. niger (Ethanol)

5. Extract by traditional method

Table no. 12 Zone of inhibition obtain from Extract acquired from traditional method

Fungal species	Concentration of extract			
	30µg/ml	50µg/ml	80µg/ml	100µg/ml
	Zone of inhibition			
aspergillus niger	9 mm	11 mm	12mm	14 mm

Fig.32 A. niger (extract by traditional method)

An antifungal study reports that laboratory-scale Soxhlet extraction yields extracts with a greater zone of inhibition than those produced by traditional methods. In soxhlet extraction Comparing between Acetone, Ethanol and Chloroform extract the chloroform extract gives greater zone of inhibition (25mm). Ethanol extract (20mm) gives the slightly greater zone of inhibition then the acetone extract (10mm).

CONCLUSION

The kokum is belong to the Species Garcinia Indica choisy, where kokum is biologically known as Garcinia Indica. Kokum oil contain Garcinol as one of the bioactive compound which is responsible for antimicrobial activity. (bioactive constituent). The garcinol is chemically polyisoprenylated benzophenone, which gives the positive response for Preliminary phytochemical screening of phenolic compound. The garcinol is mainly responsible for the antimicrobial activity.[5]The extraction of kokum oil by lab scale soxhlet extract it gives the higher yield of extract then the traditional method of extract which found to be 46.25% (except Chloroform extract). In lab scale soxhlet extraction the Acetone gives the higher yield that is 87.5% then the Chloroform and Ethanol. The yield of Chloroform extract was found to be 35.75% which is lowest yield of extract. The Ethanol shows modreate yield of extract which is 68.75%.

The Chromatographic estimation of Garcinia Indica by Thin layer chromatogaphy shows the blue fluorescent band under the UV light (254nm). The Garcinol which is benzophenone derivative which shows the blue fluorescent band under the UV light in TLC. So the estimation of the Garcinia Indica by TLC confirms the presence of garcinol in kokum extract. The extraction of kokum oil by lab scale soxhlet extract it gives greater antibacterial and anti-fungal activity then the extract obtained by traditional method. In lab scale soxhlet extraction the Chloroform gives greater antibacterial and antifungal activity then the Acetone and Ethanol. The Ethanol shows moderate antibacterial and antifungal activity (lower than Chloroform but greater than Acetone). The Acetone shows the lowest Antibacterial and antifungal activity. As the concentration of the oil extract increases the zone of inhibition for both antibacterial and antifungal activity increases.

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