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Garcino Gel: A Result of Extensive Comparative Anti-Microbial Study of Various Bioactives of *Garcinia indica*.

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ABSTRACT

Medical herb are moving from fringe to mainstream use with greater number of people seeking remedies and health approaches from side effects caused by synthetic materials. In this study, *Garcinia Indica* is various forms ie, unripe fruits, ripe and ripe dried were subjected to methanol and aqueous extraction. All extracts were screened against different bacteria and fungus by using agar well diffusion method and minimum inhibitory concentration. Aqueous extract of dried fruits along with the aqueous fraction of same extract showed very good activity against most of the micro organisms. Fraction was more potent than extract. Among antibacterial and antifungal the fraction was more potent against fungus, thus it was selected to formulate topical gel; Garcino gel. Garcino gel was successfully formulated as effective antifungal gel.

Keywords: *Garcinia Indica*, Kokum, Anti-microbial, Aqueous dried fraction, Garcino gel.

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INTRODUCTION

Antimicrobial agent is which kills or inhibit the growth of microorganisms. Nowadays synthetic antimicrobials produce serious side effects in human being and also they are prone to multi drug resistance problem. These are the alarming issues due to which the researchers are turning back to alternative system of medicine. One of the alternative systems is herbal medicine. Recently world is exploring this are due to their efficiency and minimal side effects.[1-4]

Garcinia Indica C or commonly known as Kokum and is distributed mainly in peninsular India. This is one of several species of *Garcinia* found in many tropical regions. The Kokum is a tall tropical evergreen tree. The fruit is harvested during April-May of every year. It is used as culinary in several cooking practices. The extract of the fruit has both antifungal and antibacterial properties and therefore, has a potential for use as bio-preservative in food applications [5,6]. The juice has a distinctive acidic flavor. It is a soothing drink in summer months and it provides relief from gastric disorders. It is traditionally used to treat sores, skin ailments 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 [7,8].

Garcinia Indica contain many phytoconstituents like garcinol which shows a strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β-diketone moiety and hence exerts an anti-inflammatory effect and it is also an anticancer agent [9,10]. Another major compound reported to be present hydroxy citric acid [HCA]. A naturally produced acid which reduce obesity [11]. There are studies which reports antibacterial and antifungal activity of various extract of *Garcinia Indica* fruits but none of the study depicts comparative reports on the raw, ripen and dry fruits extract as well as fractions.

Thus, we aimed at finding out the potent extract or fraction by comparative study of unripe, ripen and dry fruits extract as well as fractions of *Garcinia Indica* for their antibacterial and anti-fungal activity. The bioactive was then selected for formulation of topical gel: Garcino gel.

MATERIAL AND METHODS

Solvent and chemicals

Garcinia indica unripe, ripe and dried fruits were collected from Konkan region, Sadavali. All chemical and reagents used were of analytical grade.

Test Microorganisms

For anti-bacterial study gram positive bacteria such as *Staphylococcus aureus , Bacillus subtilis* and gram negative bacteria like *Klebsiella pneumoniae, Escherchia coli* were used in the study.

For anti-fungal study *Candida albicans* and *Aspergillus niger* were used in the study.

Preparation of extracts and fractionation of extract

All the fruits (ripe and unripe) were washed and seeds were removed. 100 gm of ripe fruits were shade dried and then used as dried fruits. Soxhlet extraction method was employed for Aqueous and alcoholic extraction of unripe, ripe and dried fruits. Each extract was filtered, evaporated and dried. Dried extracts were used for the preparation of fractions using separating funnel. Aqueous (polar) and chloroform (non polar) fractions were obtained from Aqueous extract.

Antimicrobial Study [12]

All test solutions of extracts and fractions were prepared by dissolving 500mg to 10 ml of respective solvents.

Antibacterial assay

In-vitro antibacterial activity was evaluated using the agar well diffusion technique. Nutrient agar was used as the medium. The sterile agar was inoculated with the bacteria culture for 48 hrs, at 37°C. Wells were bored by using a sterile borer and different extracts and fractions were place into them. Plates were kept for 2 hrs in the refrigerator to enable pre-diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hrs) at 37°C.

Antifungal assay

In-vitro antifungal activity was evaluated using the agar well diffusion technique. Potato dextrose agar was used as the medium. The sterile agar was inoculated with the bacteria culture for 48 hrs, at 37°C. Wells were bored by using a sterile borer and different extracts and fractions were place into them. Plates were kept for 2 hrs in the refrigerator to enable pre-diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hrs) at 37°C.

Formulation of gel [12]

Depending upon the antimicrobial activity the most efficient extract/ fraction was selected to formulate topical gel. Gel was formulated by referring to our previous work. **[**9] Weighed amount of Carbopol 940P was soaked in distilled water overnight. The bioactive extract was accurately weighed and uniformly suspended in water. The solution was then added to the soaked Carbopol. The mixture was stirred using an overhead stirrer for around 1-1.5 hours; till a uniform suspension was obtained. Care was taken to prevent air entrapment during stirring. This was followed by neutralization of the gelling agent by NaOH or 50% triethanolamine; pH was adjusted to 7.3-7.5. The composition of gel formulation (Garcino gel) given in Table 5.

Evaluation of Gel [12]

Formulations were evaluated for various parameters such as appearance, color, pH, viscosity, Homogeneity, spreadability, Primary dermal irritation index.

Viscosity

The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA).

Homogeneity

Homogeneity of formulated gels was examined by visual inspection for presence of any aggregates.

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreabability was calculated using the formula S = M.L / T, Where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

Primary dermal irritation index (PDII)

This test is done by applying the formulated gel on to the skin and then observed for any reversible damage to the skin within 4 hrs. Based on their PDII score, the formulation can be graded as irritating or non-irritating.

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as an average of three analyses ± standard deviation. Statistical analyzes was performed by the t-test.

RESULT AND DISCUSSION

Antimicrobial study

As discussed in the table 1 and 2 we carried out preliminary study for anti bacterial and antifungal activities on six extracts. Aqueous and methanol extract of each unripe, ripe and dried and it was observed that aqueous dried extract show better activity compared to others.

Study was further extended to the fractions and two fractions of each type were analyzed for their anti-microbial action. All fractions except chloroform exhibited comparatively good activity than their respective extract (table 3 & 4). Among all fractions aqueous fractions of dried fruits showed very potent antibacterial and antifungal activity against most of organisms (Fig 1 & 2).

When the effect was compared of all extracts and fractions on different organisms we found that aqueous dried extract as well as fraction were most effective. At 3 mg of concentration, as compared to aqueous extract, aqueous fraction of dried fruit showed double the zone of inhibition (Fig 3). This effect may be due to the removal of inactive polar compounds by fractionating with chloroform.

It was found that kokum is more effective as anti fungal agent then anti bacterial. Thus, we expanded our work to formulate a topical gel; Garcino gel which will be effective against skin diseases. We selected the aqueous fraction of dried fruit which is most potent, to formulate Garcino gel.

Evaluations of Formulations

The aqueous fraction of dried kokum fruit was formulated into a gel; Garcino-gel did not show a considerable change in characters like color, odor, and consistency, and there was no phase separation observed during the course of the study.

The results of pH, spreadability, and viscosity of the formulations are recorded in Table 6. The result depicted that Garcino-gel is compatible with the skin, well viscous to spread and to retain on to the skin. The extrusion from the tube and spreadability of the topical formulation is important during the application, as also patient acceptance. The Garcino-gel showed acceptable spreadability along with good extrusion. As, the Garcino-gel was found to be the best among all the combination, it was carried forward for microbial contamination test and antimicrobial study.

Antimicrobial Activity of Garcino gel

Table 7 shows the antimicrobial activity of the Garcino gel. Garcino gel emerged as very good effective antifungal medicine. All the experiments were performed in triplicates.

CONCLUSION

The detailed comparative study between unripe and ripe extract and fraction gave a good insight of kokum fruit potential as antimicrobial agent. The fractionation process produce a very potent fraction (aqueous dried) as more antifungal agent than anti bacterial. Thus, we could successfully formulate Garcino gel containing this aqueous fraction. When science leads to final marketed product it serves better for the upliftment of society. Thus, we conclude that our work will be a one of the progressive step for building up the market of kokum in the world. This in turn will favor to grow the economy of Konkan people as Kokum is a crop of Konkan region.

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Table 1: Comparative study of Antibacterial activity of various Garcinia extracts at three different concentrations

	E. C			B.S			S.A			K.P		
Concentration Extract	20μl 1mg	40 μl 2 mg	60 μl 3 mg	20 μl 1 mg	40 μl 2 mg	60 μl 3 mg	20 μl 1 mg	40 μl 2 mg	60 μl 3 mg	20 μl 1 mg	40 μl 2 mg	60 μl 3 mg
Aqueous Unripe fruit	0	0	1.3	-	1.4	2	0.8	1.5	1.8	1	1.3	2
Methanol Unripe fruit	0	0	0	0	0	0	0	0	0	0	0	0
Aqueous ripe fruit	0	0	0	0	1.3	1.8	1	1.3	1.5	1.5	1.8	1.9
Methanol Ripe fruit	0	0	1.1	1	1.2	1.6	1	1.4	1.7	1	1.5	2
Aqueous Dried fruit	1	1	2	3	3.1	3.5	2	2.3	2.5	2.5	3	3
Methanol Dried fruit	0	0	0	0	0	0	0	0	0	0	0	0

Microorganisms used E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus and* K.P - *Klebsiella pneumoniae.* Extract volume used are 20 µl, 40 µl and 60 µl. Concentration of Extract 1mg, 2mg and 3mg

Table 2: Comparative study of Anti-fungal activity of various Garcinia extracts at three different concentrations

	Aspergillus niger			*Candida albicans*		
Concentration Extract	20 μl 1mg	40 μl 2 mg	60 μl 3 mg	20 μl 1mg	40 μl 2 mg	60 μl 3 mg
Aqueous Unripe fruit	2	3.3	3	2	2.3	3
Methanol unripe fruit	1	1.3	1	0	0	0
Aqueous ripen fruit	3	3.5	4	2.3	3.5	4
Methanol Ripen fruit	2	3	3	2	2.5	3.5
Aqueous Dried fruit	4.6	4.8	6	4	4.6	6
Methanol Dried fruit	5	4.8	5	4	5	5

Microorganisms used E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus and* K.P - *Klebsiella pneumoniae.* Extract volume used are 20 µl, 40 µl and 60 µl. Concentration of Extract 1mg, 2mg and 3mg

Table 3: Comparative study of Antibacterial activity of various Garcinia fractions at three different concentrations

	E.C			B.S			S.A			K.P		
Concentration Fractions	10 μl 0.5 mg	20 μl 1 mg	30 μl 1.5 mg	10 μl 0.5 mg	20 μl 1 mg	30 μl 1.5 mg	10 μl 0.5 mg	20 μl 1 mg	30 μl 1.5 mg	10 μl 0.5 mg	20 μl 1 mg	30 μl 1.5 mg
Aqueous Unripe fruit	1.5	2.1	2.7	2	2.5	3.1	1.5	2.1	2.8	2	2.5	3.1
Chloroform unripe fruit	0	0	0	1.3	1.8	2.2	1.5	2.0	2.6	0	0	0
Aqueous ripe fruit	1	1.4	2.2	2.7	3.3	3.8	2	2.6	3.3	2.5	3.1	3.7
Chloroform ripe fruit	0	0	1	0	0	0	0	0	0	0	0	0
Aqueous Dried fruit	2	2.6	3.2	2.5	3.1	3.6	2.6	3.2	3.8	2.6	3.2	3.6
Chloroform Dried fruit	1	1.5	1.8	1	1.2	1.4	2.5	2.9	3.2	1	1.1	1.4

Microorganisms used E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus and* K.P - *Klebsiella pneumoniae.* Fraction volumes used are 20 µl, 40 µl and 60 µl. Concentration of fractions 0.5mg, 1mg and 1.5mg

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Table 4: Comparative study of Antifungal activity of various Garcinia fractions at three different concentrations

	Aspirgillus niger			*Candida albicans*		
Concentration Fractions	**10 μl 0.5 mg**	**20 μl 1 mg**	**30 μl 1.5 mg**	**10 μl 0.5 mg**	**20 μl 1 mg**	**30 μl 1.5 mg**
Aqueous Unripe fruit	**1**	**1.5**	**3**	**2**	**2**	**2**
Chloroform unripe fruit	**0**	**0**	**0**	**0**	**0**	**0**
Aqueous ripe fruit	**1**	**1**	**2**	**1**	**2.2**	**1.3**
Chloroform ripe fruit	**0**	**0**	**0**	**0**	**0**	**0**
Aqueous Dried fruit	**4**	**4**	**5**	**3**	**5**	**4.8**
Chloroform Dried fruit	**0**	**0**	**1**	**0**	**1.5**	**0**

Microorganisms used AN - *Aspirgillus niger and* CA - *Candida albicans.* Fraction volumes used are 20 µl, 40 µl and 60 µl. Concentration of fractions 0.5mg, 1mg and 1.5mg

Table 5: Combination of ingredients for formulation of gel

Ingredients	**Formulation code**
	A
Aqueous fraction	3 %w/w
Carbopol 940P	2 %w/w
Flavour	q. s
Water q. s	100gm
50 % Triethanolamine	pH 7.3-7.5

Table 6: Evaluation parameters of formulated gel

Sr.no	**Parameters**	**Observations**
1.	Appearance	Lustrous
2.	Color	Pale yellow- white
3.	pH	7.3-7.5
4.	Viscosity	5998 ± 152 cps
5.	Spreadability	6.9 4± 1.21 secs

All the experiments were performed in triplicates

Table 7: Anti-microbial study of Garcino Gel

Organisms	**Zone of inhibition (cm) mg Gel (containing 3 mg Aqueous dried fraction/well)**
Escherichia coli	3.1
Bacillus subtilis	3.4
Staphylococcus aureus	3.3
Klebsiella pneumoniae	3.5
Aspergillus niger	5.1
Candida albicans	4.9

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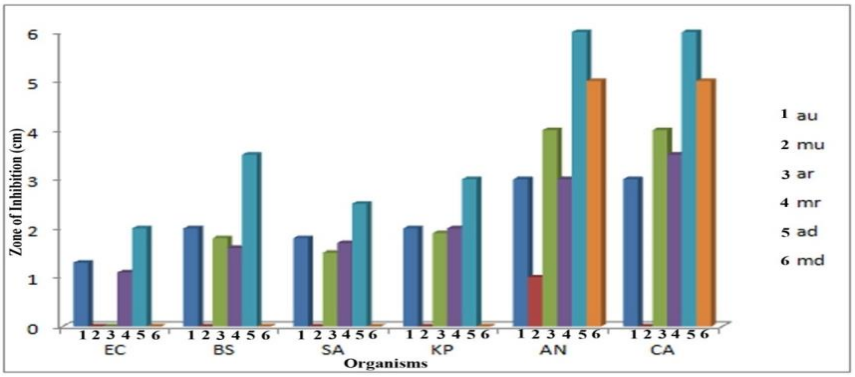
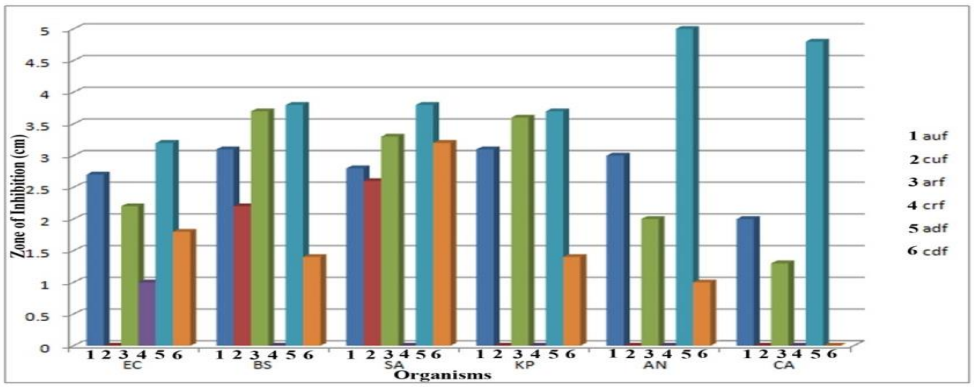
Fig 1: Graphical Representation of Antimicrobial and Antifungal activity of all Garcinia fruit extract.



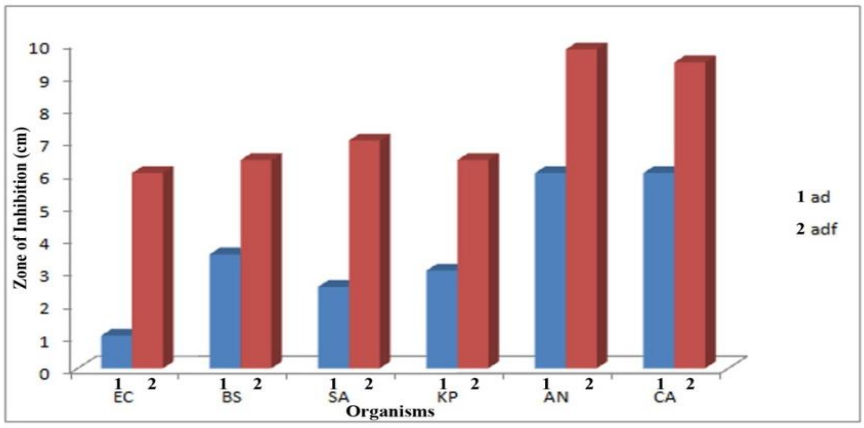
Microorganism used are E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus,* K.P - *Klebsiella pneumoniae,* AN - *Aspirgillus niger and* CA - *Candida albicans.* Extract used are au- Aqueous Unripe fruit extract, mu- Methanol Unripe fruit extract, ar - Aqueous ripe extract, mr -Methanol Ripe fruit extract, ad- Aqueous Dried fruit extract, and md- Methanol Dried fruit extract at volume 60μl. Concentration of extract 3mg

Fig 2: Graphical Representation of Antimicrobial and Antifungal activity of all Garcinia fruit fractions.



Microorganism used are E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus,* K.P - *Klebsiella pneumoniae,* AN - *Aspirgillus niger and* CA - *Candida albicans.* fraction used are auf- Aqueous Unripe fruit fraction, cu- chloroform Unripe fruit fraction, arf - Aqueous ripe fraction, crf -chloroform Ripe fruit fraction, ad- Aqueous Dried fruit fraction and cd- chloroform Dried fruit fraction at volume 30μl. Concentration of extract 1.5mg

Fig 3: Graphical Representation of Antimicrobial and Antifungal activity of aqueous dried Garcinia fruit extract and fractions at 60μl



Microorganism used E.C - *Escherchia coli,* B.S - *Bacillus subtilis,* S. A. -*Staphylococcus aureus,* K.P - *Klebsiella pneumoniae,* AN - *Aspirgillus niger and* CA - *Candida albicans. Extract volume 60 μl. Concentration of Extract used 3mg.*

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