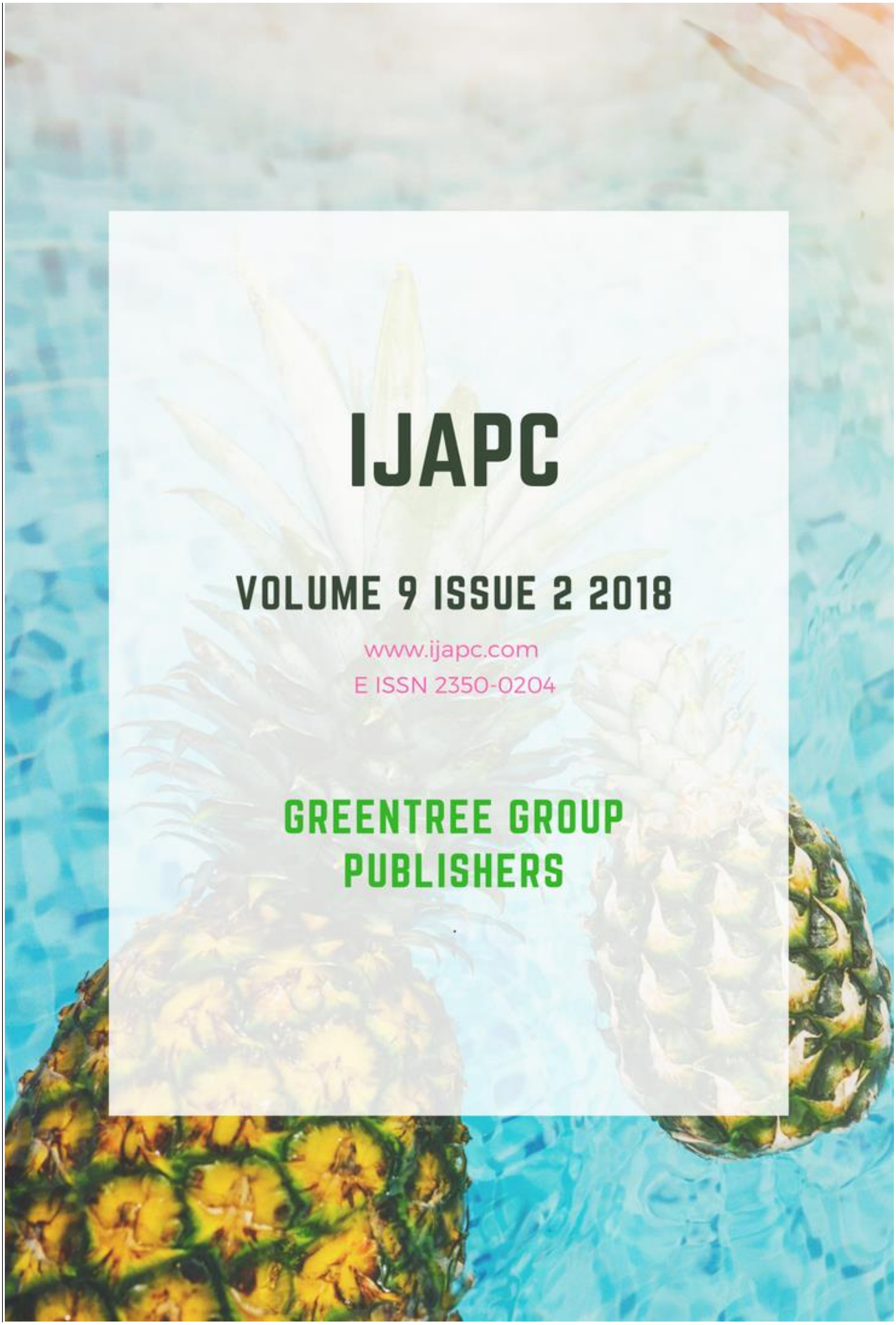

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# Testing the Anti-microbial Activity of the Extracts of Dried *Kokum* (*Garcinia indica*) as a Potential Bio-preservative

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## ABSTRACT

*Garcinia indica* has been used by local people of the Konkan region to cure perishable food such as fish and meat as a part of traditional culinary practice. The antimicrobial activity of the plant extract is proven in the scope of developing herbal formulations for therapeutic usage. With an aim to minimize the usage of chemicals in storage of cereals, pulses, etc.; efforts were made to establish the potential of *Garcinia indica* extract as a bio preservative for storage of dry staples used in cooking. The current work focuses on preparation of the extract in a suitable ash as a product and it's evaluation as a preservative against representative dry staples found in regular culinary use. The antimicrobial activity of the prepared ash was checked and it was found that it can be a potential bio preservative for disinfection and storage of dry staples such as rice, dal, groundnuts etc.

## KEYWORDS

*Antimicrobial, Herbal, Garcinia indica, Kokum, Bio preservative*

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## INTRODUCTION

In many Indian households, dry staples used in cooking such as rice, dal, etc. are bought in bulk and stored in containers for 6-12 months and used in cooking. This is done as dry staples are a necessity and buying them in bulk proves to be cost effective. However, spoilage of dry goods is seen during change of seasons as moisture accumulates in such bulk stored goods. The food products are generally sun-dried prior to their storage.

In addition to this, chemical preservatives such as boric acid powder are added to the sun dried dry staples prior to storage. The purpose is generally to preserve the natural characteristics of food and to increase the shelf life of food, and inhibit natural ageing and discoloration that can occur during food preparation. Boric acid is reported to be used as food preservatives in some foods and food products [1]. This is because boric acid is able to inhibit the growth of microorganism; therefore, the preserved food can stay fresh and last longer.

However, since boric acid contains cumulative toxicity, FAO/WHO Expert Committee has declared that boric acid is unsafe to use as a food additive. Yet, many Indian households use boric acid powder to preserve dry staples used in cooking.

One of the major reasons for deterioration of food products during processing and storage is lipid peroxidation. The addition of antioxidants is a method of increasing the shelf-life, especially of lipids and lipid containing foods. Synthetic antioxidants have restricted use in foods as they are suspected to be carcinogenic. Hence, the importance for search of natural alternatives especially of plant origin has greatly increased in recent years [2].

The objective of this study was to investigate the use of plant extract of *Garcinia indica* as a potential bio-preservative for storage of dry goods. This may lead to the discovery of an alternative form of treatment other than chemical preservatives being used at present, to which many of the bacteria are developing resistance. Many plants have been used traditionally to preserve perishable food items and researchers have noted that further investigations on these plants might lead to the development of antimicrobial preservatives.

## MATERIALS AND METHODS

*Garcinia indica* rinds, hot plate, distilled water, copper based pot, spatula, muslin cloth, beaker, hot air oven, evaporating dish, muffle furnace, silica crucible, clean dry sterile 5 ml, 2 ml. 1 ml pipettes, sterile

Nutrient agar media, sterile saline, sterile nutrient broth, clean dry sterile suspension tubes, conical flasks, distilled water, colorimeter, incubator, Bunsen burner, st. Petri plates, *Garcinia indica* ash, rice, groundnuts, Pigeon Pea (Toor dal), glass bottles, water.

## EXPERIMENTAL

Preparation of *Garcinia indica* extract:

Dried *Garcinia indica* rinds, 50 g was introduced into 500 ml Distilled water and the solution was heated on a hot plate till the volume of the extract was reduced by half (250 ml). The liquid was filtered with a muslin cloth and further evaporated on the hot plate until dryness was achieved. Thus, *Garcinia indica* extract was prepared using the hot maceration technique. The extract was kept for complete drying until formation of crystals in the hot air oven at 105°C for 4 hours and then at 60°C for minimum three days. The crystals were scraped and used for further analysis.

Initially, the crystals of *Garcinia indica* were decided as the product to be tested for its bio preservative potential. However, due to the hygroscopic nature of the crystals, the formation of the crystals into ash was considered in order to prevent loss of bio-activity and also for convenient usage.

Preparation of Ash:

Dried crystals, 1 g were placed in a suitable silica crucible previously cleaned and weighed. The crystals were spread evenly and accurately. The materials were incinerated by gradually increasing the heat, not exceeding at 450°C until free from carbon, cooled in a desiccator and used for further analysis. **Fig 1** "Ash of *Garcinia indica* rinds".

**Fig 1** "Ash of *Garcinia indica* rinds"

Anti-microbial activity of the *Garcinia indica* ash against bacterial strains:

The microorganisms used were *Staphylococcus aureus* and *Escherichia coli.* These bacteria have been extensively implicated in food poisoning. Fresh bacterial cultures were prepared by sub-culturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37°C for 24 hours. These 24-hour-old bacterial cultures were transferred into freshly prepared nutrient broth and standardised to 0.1 OD by $OD_{600}$ method[3] using a colorimeter.

A protocol was devised and subsequent additions were made as per the following

table for assessing the anti-microbial activity of the ash against the bacterial cultures:

An ash suspension was prepared by dissolving 0.50 gm of ash in 2 ml distilled water and 1 ml of this suspension was added in tubes 1 and 2 for *E. coli* and *S. auerus* sets, respectively. **Table 1** "For *E. coli"* and **Table 2** "For *S. auerus"*.

**Fig 2** "Day One Groundnuts"

**Fig 3** "Day one Toor Dal"

**Fig 4** "Day One Rice"

**Table 1** Turbidity measurement for *E. coli*

| | |
|---|---|
| **Tube 1** | 1 ml ash suspension + 0.5 ml culture + 5 ml media |
| **Tube 2** | 1 ml ash suspension + 0.5 ml culture + 5 ml media |
| **Tube 3** | 0.5 gm salt + 0.5 ml culture + 5 ml media |
| **Tube 4** | 0.25 gm ash + 0.5 ml culture + 5 ml media |
| **Tube 5** | 0.25 gm ash + 0.5 ml culture + 5 ml media |
| **Positive Control** | 0.5 ml st. saline + 0.5 ml culture + 5 ml media |
| **Negative Control** | 1 ml st. saline + 5 ml media |
| **Negative Control** | 1 ml st. saline + 5 ml media + 0.1 gm ash |

**Table 2** Turbidity measurement for *S. auerus*

| | |
|---|---|
| **Tube 1** | 1 ml ash suspension + 0.5 ml culture + 5 ml media |
| **Tube 2** | 1 ml ash suspension + 0.5 ml culture + 5 ml media |
| **Tube 3** | 0.5 gm salt + 0.5 ml culture + 5 ml media |
| **Tube 4** | 0.25 gm ash + 0.5 ml culture + 5 ml media |
| **Tube 5** | 0.25 gm ash + 0.5 ml culture + 5 ml media |
| **Positive Control** | 0.5 ml st. saline + 0.5 ml culture + 5 ml media |
| **Negative Control** | 1 ml st. saline + 5 ml media |
| **Negative Control** | 1 ml st. saline + 5 ml media + 0.1 gm ash |

The results from the above analysis were positive and bottling studies were performed to observe the anti-microbial activity of ash against dry staples.

A protocol was devised as seen in **Table 3** "Protocol". The samples were visually observed and results were recorded at the interval of one week. Fungus infestation prior to analysis was observed. (**Fig 2** "Day

one groundnuts", **Fig 3** "Day one toor dal", **Fig 4** "Day one rice").

**Table 3** Bottling Assay

| | **Lipids (Groundnut)** | **Carbohydrate (Rice)** | **Proteins(Pigeon Pea)** |
|---|---|---|---|
| **Set 1** | Plain groundnut + Ash (Control) | Plain Rice + Ash (Control) | Plain Pigeon Pea + Ash (Control) |
| **Set 2** | Fungus infested (ash treatment) | Fungus infested (ash treatment) | Fungus infested (ash treatment) |
| **Set 3** | Plain groundnut (fungus infestation after one week) | Plain rice (fungus infestation after one week) | Plain pigeon pea (fungus infestation after one week) |
| **Set 4** | Fungus infested (no treatment) | Fungus infested (no treatment) | Fungus infested (no treatment) |

Note: Observation began once fungus infestation took place. Observation of the samples was carried out after seven days (**Fig 5** "Decrease of fungus in toor dal", **Fig 6** "Decrease of fungus in rice", **Fig 7** "Decrease of fungus in groundnuts"). **Fig 5** "Decrease of fungus in Toor Dal"

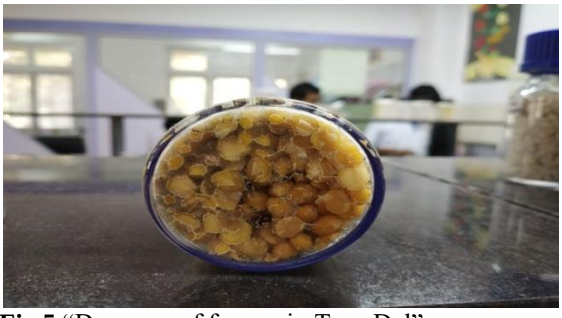

**Fig 5** "Decrease of fungus in Toor Dal"

**Fig 6** "Decrease of fungus in rice"


**Fig 7** "Decrease of fungus in ground nuts"

The samples pre-treated with ash showed no change upon observation after a week (**Fig 8** "Pre-treated samples showing no visible change (Day 7)". In addition, a set of samples were kept as a control to observe the visible change, i.e no ash treatment was done on these samples after fungus infestation (**Fig 9)** "Non treated grounduts", **Fig 10** "Non treated toor dal", **Fig 11** "Non treated rice").


**Fig 8** "Pre-treated samples showing no visible change (Day 7)"

**Table 4** Results of turbidity measurement

| Absorbance at 600 nm | Analysis carried out on 08.03.2018 | Analysis carried out on 16.03.2018 |
|---|---|---|
| Positive Control (*E. coli*) | 0.42 | 0.40 |
| Positive Control (*S. auerus*) | 0.38 | 0.39 |
| Negative Control | 0.00 | 0.00 |
| Tube 1 (*E. coli*) (Ash suspension) | 0.10 | 0.12 |
| Tube 2 (*E. coli*) (Ash suspension) | 0.17 | 0.15 |
| Tube 1 (*S. auerus*) (Ash suspension) | 0.12 | 0.10 |
| Tube 2 (*S. auerus*) (Ash suspension) | 0.10 | 0.13 |
| Tube 1 (*E. coli*) (Direct Ash) | 0.02 | 0.02 |
| Tube 2 (*E. coli*) (Direct Ash) | 0.03 | 0.01 |
| Tube 1 (*S. auerus*) (Direct Ash) | 0.05 | 0.04 |
| Tube 2 (*S. auerus*) (Direct Ash) | 0.04 | 0.04 |
| Salt (*E. coli*) | 0.22 | 0.20 |
| Salt (*S. auerus*) | 0.24 | 0.22 |

## RESULTS AND DISCUSSION

An application of Ash obtained from *Garcinia indica* fruit rinds was developed for the first time as preservative for food products. Anti-microbial activity of the same was demonstrated to elucidate the mechanism of the herbal preservative. As stated in the **Table 4** "Results of turbidity measurement" there is a considerable decrease in the turbidity of the suspension. In spite of desalting of rinds, Salt was kept as a control to shun the interference of the residual salt.

**Fig 9** "Non-treated groundnuts"

**Fig 10** "Non treated Toor Dal"

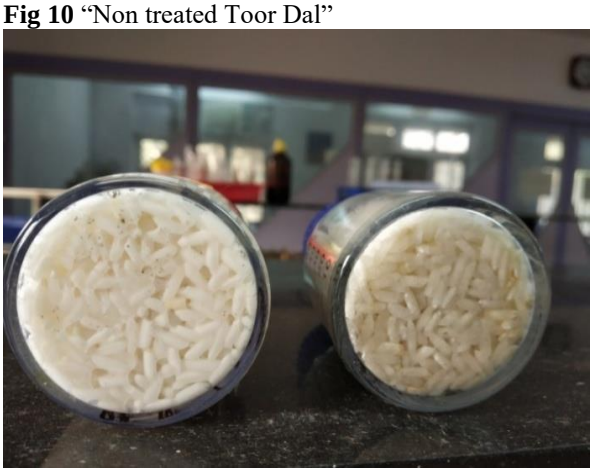

**Fig 11** "Non-treated Rice"

From the current study it was evident that the addition of direct ash shows substantial antimicrobial activity against the bacterial culture.

For the real time study of preservative action of Ash, 35 g of grain samples were employed. The curative and preventive property of the ash was represented by the significant decrease in fungus or no growth observed in samples kept for evaluation.

## CONCLUSION

The potential of the *Garcinia indica* ash as a bio preservative was studied in this research project for the first time. The anti microbial activity of *Garcinia indica* is previously reported[4] for its therapeutic use but the anti microbial activity of the extract as a food application was studied for the first time. Due to the hygroscopic nature of *Garcinia indica*, an ash was prepared and this product was developed for its use as a bio preservative. After establishing the anti microbial activity of the new product against common food borne bacteria, the product was tested against representative samples of dry staples commonly found across all households in India. It is commonly used as a preservative for storing perishable items such as fish, meat in traditional Indian cooking practices. An attempt was made at using *Garcinia indica* in the form of ash for storage of rice, dal, and pulses for the first time. The anti-microbial activity of the developed ash was appreciable as found in the turbidity measurement. As the turbidity measurement was significantly lower in the tubes containing ash, the ash was deemed of a good quality and was used for further analysis.

The ash was further tested on dry goods representing major macromolecular groups namely Proteins, Carbohydrates and Fats. The specimens selected were Pigeon Pea, Rice and Groundnuts. This analysis was carried out in two perspectives: preventive and curative. A set of samples which was fungus infested was added with ash and kept for observation to study the product's curative property. An appreciable lowering of fungus was observed in this set of samples. Likewise, water was externally added to induce fungal growth to a set of samples previously treated with ash. No significant growth of ash was observed in this set of samples. The product shows preventive property for fungal growth. A future prospect of this research would be to perform aflatoxin study to check the chemical remnants of fungus in the samples. The product shows curative and preventive efficiency but the recommended usage of the product would be its use as a preventive measure for long term storage.

The developed ash is non toxic as no metals/ toxic reagents were used in the development process. The samples were washed under tap water and the ash was

removed from the samples making the samples safe for consumption. However, 50 g of dried rinds can produce only 3-4 gm of ash. Hence, analysis has to be done to increase the yield of ash in order to scale up the product for routine use. Long term shelf life studies should be carried out (3-6 months) in order to determine absolute efficacy of the developed product.

Thus, *Garcinia indica* ash can be used as a bio preservative for storage of dry goods.

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